THE UNIVERSITY OF ALBERTA

FOOD POISONING POTENTIAL OF BOLOGNA IN SANDWICHES

by

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A THESIS

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ABSTRACT

The incidence of pathogenic bacteria in vacuum packaged bologna is low, with coagulase positive S. aureus confirmed in 0.9% of samples (100 per gram) and clostridia detected at levels up to 160 per gram in 9.0% of samples. Salmonellae and E. coli type I were not detected. Non-pathogenic microflora and pH were variable. Lactic acid bacteria and Group D streptococci were detected most frequently. Group D streptococcus counts and comparison of total counts at 4°, 21° and 36°C suggest that 29-60% of bologna samples had been exposed to storage temperature abuse. The microflora and pH were affected by manufacturer and age, and to some extent by display type.

Pathogenic bacteria, except C. perfringens, inoculated at low concentrations (4.4×10^1 to 1.6×10^3 per gram) onto bologna in sandwiches were capable of significant growth only after more than 8 hours incubation at 30°C. C. perfringens failed to develop on any bologna. All other pathogens, except S. aureus, were inhibited on bologna having pH below 6.1. S. aureus exhibited reduced growth on bologna at pH 5.5 and was completely inhibited at pH 5.1. Only Gram negative pathogens were affected adversely by increased competition.

These results indicate that bologna in sandwiches could be a vehicle of food poisoning only in certain, almost

unrealistic (more than 8 hours at 30°C), situations. Combinations of inhibitors appear to be responsible for this safety. The results also indicate that safety of vacuum packaged bologna is not affected adversely by high saprophyte counts.

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Chapter I. Introduction

Each day in North America, sandwiches are consumed in enormous numbers. Bologna is an inexpensive and widely accepted processed meat product which, therefore, may be extensively used in sandwiches. Prior to consumption, sandwiches may be subjected to storage abuse at temperatures up to 30°C or higher (e.g. on a warm summer day). Picnic baskets, automobiles, lunch boxes on construction sites, passenger trains, school lockers and desk drawers are only a few of the common "incubators". Sandwiches may be prepared in the home from 4 to 18 hours or more prior to intended consumption time, and may then be "forgotten" and eaten later, thus extending the incubation period. Catering companies may store sandwiches at ambient temperatures for 36 to 96 hours before sale (1,84). Thus, time and temperature are suitable for growth of pathogens in sandwiches.

Before growth can occur, there must be contamination. Humans carry a variety of pathogenic microorganisms on their hands. Handling of ingredients during preparation of sandwiches probably results in low levels of pathogen contamination. Sandwich fillings may represent a suitable growth medium for pathogens. Longree *et al.* (84) suggested that protein based sandwiches exposed to storage abuse were potentially dangerous. Bologna, therefore, may be a suitable

growth medium for pathogenic bacteria.

This simple theory suggests sandwiches as probable vehicles of food poisoning (8). However, there are few reported cases of sandwich-borne food poisonings (1,87). This may be the result of individuals not reporting cases of food poisoning. Bryan (14) stated that only a small fraction of food-poisonings are reported. On the other hand, the low incidence of food poisonings attributed to sandwiches may be due to pathogens failing to grow.

The primary objective of this study was to determine the food poisoning potential of bologna sandwiches. To relate the study to practical conditions, the bacterial quality of vacuum packaged, sliced bologna offered for sale in the Edmonton retail marketplace also was determined.

Chapter II. Literature Review

The sandwich filling is cited (84) as the most hazardous component of sandwiches. Hence, literature concerning the microbiology and manufacture of bologna and related products are considered in this review.

1. Bologna Formulation and Processing

The term "sausage" can be used to describe a variety of different products which can be classified into smaller groupings according to methods of manufacture. Sausage may be of the ground or emulsion variety; fresh or cooked; and fermented or non-fermented. Sausage may be classified further according to the ingredients used in the formulation, or the size of the finished product [eg. bologna and frankfurters are basically the same product (9)].

Bologna is a cooked, cured, smoked, emulsion-type, non-fermented sausage. It has been suggested that bologna is the most universally used sausage in the U.S.A. (136). Bologna formulations vary slighty between manufacturers and even between different batches from the same manufacturer. Appendix I lists 3 possible formulations. All formulations include certain basic ingredients: beef, pork, spices, salt and nitrite. In addition, sugar, skimmilk powder, cereal, and certain additives (e.g. ascorbate or erythorbate) are

used by many manufacturers (43,82).

Production of bologna involves the series of unit operations outlined below (9,43,82,136). Initial operations involve grinding meat chunks and mixing the other ingredients to obtain a uniform blend. The emulsion is then formed by chopping the blend until meat proteins (primarily myosin) are solubilized and fat particles are suspended in the protein solution. Often, ice or cold water is added during chopping to prevent temperature increases and to impart good flow characteristics to the emulsion. The emulsion is then stuffed into casings. Prior to smoking, bungs (i.e. stuffed casings) may be allowed to dry for a short time (e.g. 1 hour).

Bungs are smoked, or smoked and cooked for 6-10 hours. Initial smoking temperatures are 43-53°C. The temperature should increase 5-7°C per hour until a temperature of 77°C (internal product temperature 70°C) is attained. Myosin protein coagulates at 55°C and collagen begins to shrink near 65°C. If bologna is heated too rapidly, the surface of the bung will set and begin to shrink while the interior is still liquid, causing the bung to split. Following smoking and cooking, bologna is given a cold shower and is held briefly (e.g. 30 minutes) at room temperature before being placed under refrigeration. Bologna may be distributed to retailers as whole bungs, or may be sliced and vacuum

packaged prior to distribution.

2. Microflora of Bologna and Related Products

When considering the microflora of any food product it is important to differentiate between normal saprophytic flora, potentially pathogenic flora, and indicator organisms. A certain population of saprophytic microorganisms is expected and, in many cases, is desirable in non-sterilized foods. The presence of pathogenic organisms, even in low numbers, is undesirable. Indicator organisms can provide information on the sanitation and storage conditions to which the product has been exposed. Consideration will be given to the occurrence and significance of these general categories of microorganisms in bologna and related products.

A. The Normal Saprophytic Flora

As stated, non-sterile foods contain significant numbers of saprophytic microorganisms. Bologna, although heated to 70-72°C, is not sterile. Many heat resistant organisms survive. Graham and Blumer (50) cited Bacillus as the most common genus in conventionally processed meat. Heiszler et al. (62) reported that curing agents, smoking and heat treatment selected Gram positive organisms (i.e. Micrococcus, Sarcina, Lactobacillus, Microbacterium and Bacillus) as the predominating flora in frankfurters.

Microbacteria, including M. thermosphactum, predominated in slime spoiled, cured meats (4) and spoiled, vacuum packaged fresh meat (40,110).

Bologna is subject to post-processing contamination during slicing and packaging. Slicing is the most important source of contamination and is responsible for the majority of pathogenic and saprophytic organisms in vacuum packaged, cooked, sliced meat (77,93). The variability of this contamination, especially Lactobacillus, may account for much of the conflicting information about the predominant microflora of vacuum packaged cooked meats (70). Lactobacilli, streptococci, microbacteria, micrococci and coryneforms have all been reported as predominant microflora (3,77,93,97,102,119,122,139).

The characteristic composition of the microflora after storage is not necessarily related to the composition of the microflora of the freshly packaged product. Kempton and Bobier (77) reported that most bacteria on freshly packaged, cured meats died during storage, and that lactic acid bacteria predominated after 2 weeks. Allen and Foster (2) found that catalase positive cocci predominated in the initial flora, but catalase negative organisms, classified as lactic acid bacteria, predominated after 10 days at 7°C or after 20 days at 1.1°C. Alm et al. (3) reported that, after storage, the microflora of weiners and salt-cured meat

changed from a mixed population of Bacillus, Achromobacter, and Lactobacillus to an almost pure Lactobacillus population.

The bacterial population which can be expected in vacuum packaged, cooked, cured meat appears to be related to the age of the product. Surkiewicz et al. (126) reported that 90% of frankfurters, at the time of manufacture, had a total count of less than $2x10^4$ organisms per gram. Allen and Foster (2) and Steinke and Foster (122) observed initial counts of 10^3 organisms per gram in vacuum packaged, sliced, processed meats. Warnecke et al. (139) found that the counts of vacuum packaged bologna varied from 10^3 organisms per gram after 1 day, to 10^5-10^6 organisms per gram after 5 days, to 10^6-10^7 organisms per gram after 15 days, to a maximum of 10^7 organisms per gram after 20 days storage at 9°C. Hill et al. (63) found that total counts in vacuum packaged luncheon meats, including bologna, did not rise above 10^6 organisms per gram until after 24-28 days of storage at 3-4°C. Allen and Foster (2) reported a maximum stationary population of 10^8 organisms per gram in bologna stored at 7.2°C for 40 days. Kempton and Bobier (77), Hill et al. (63) and Ingram (70) also reported maximum populations of 10^8 organisms per gram after refrigerated storage.

Attainment of maximum bacterial population does not

mean that a product is spoiled. Spoilage of vacuum packaged bologna is a function of acid by-product accumulation and not of bacterial growth (77). In fact, acid accumulation is considered by some (63,77) to impart desirable flavor to bologna. Hill et al. (63) reported that consumer acceptance of bologna increased with bacterial number (up to 10^8 organisms per gram) and that quality did not begin to deteriorate until after 46-60 day storage at 3-4°C. Kempton and Bobier (77) reported that commercial bologna stored for 15 weeks at 5°C remained acceptable in odor, appearance and flavor, although the pH dropped from 6.5 to 5.0 during storage. Allen and Foster (2) found that vacuum packaged bologna had a shelf-life of 70-80 days at 1.1°C and 40 days at 7.2°C. Steinke and Foster (122) reported that bologna stored at 5° and 10.5°C remained normal in appearance for 15 weeks. In contrast, Warnecke et al. (139) observed that bacterial growth in vacuum packaged bologna stored at 9°C adversely affected organoleptic quality.

The saprophytic microflora of bologna also influence survival and growth of pathogens. These effects will be discussed later.

B. Pathogenic Microorganisms

The presence of pathogenic organisms in vacuum packaged bologna may result from pathogens surviving heat processing or contamination during slicing and packaging. The

occurrence and fate of inoculated pathogens (e.g. S. aureus, salmonellae, enteropathogenic E. coli, B. cereus and C. perfringens) in vacuum packaged, cooked, cured meats will be reviewed.

Riemann et al. (105) reported that S. aureus was a natural contaminant of semi-preserved meats. Eddy and Ingram (36) found S. aureus in vacuum packaged bacon. On the other hand, Surkiewicz et al. (126) reported that 93% of frankfurters produced in inspected establishments were S. aureus negative. Heiszler et al. (62) reported that only 1.0-1.5% of frankfurters were S. aureus positive after smoking. Jay (74), in a survey of "non-frozen market cuts", reported all 10 samples of bologna S. aureus negative.

Several inoculation studies have also been conducted. Thatcher et al. (133) demonstrated enterotoxin production in vacuum packaged bacon which had been inoculated with 10^6 S. aureus per gram and stored at 37°C. Ingram (70) noted that S. aureus inoculated into vacuum packaged bacon increased from 10^3 organisms per gram to 10^4-10^5 organisms per gram after incubation at 25°C for 2 or more days. Genigeorgis et al. (42) observed enterotoxin B production in hams inoculated with 10^3-10^6 organisms per gram and stored at 10°, 22° and 30°C for up to 16 weeks. Toxin production was greater at 30°C than at 22° or 10°C. It appears that S. aureus can grow and produce toxin in vacuum packaged, cured

meats.

Salmonellae are rarely observed in processed or cured meats (70,102,105). Weissman and Carpenter (140) failed to isolate salmonella in 8 samples of frankfurters, but found salmonellae in 1 of 11 samples of smoked pork sausage. Khan and McCaskey (79) determined 49 bologna and lettuce sandwiches to be salmonellae negative. Surkiewicz et al. (126) found 100% (690 samples from 40 manufacturers) of frankfurters to be salmonella free. Possibly contrary to the above, Taylor and McCoy (130) reported that 8% (24 outbreaks) of salmonellosis in England and Wales between 1949-63 involved unspecified types of sausage.

The low incidence of salmonellae in cooked, cured meat could result from either destruction during processing, low incidence of post-processing contamination or poor survival of these organisms in the finished product. Inoculation studies help to clarify the behavior of salmonellae. Davidson and Webb (33) reported that S. typhimurium survived for several weeks at 7°C, and multiplied slightly after 1 week or more at 18° and 24°C, when inoculated into vacuum packaged bologna (pH near 6.0) at levels of 10^1-10^3 organisms per gram. Goepfert and Chung (45) observed a decrease in S. typhimurium inoculated into thuringer sausage at levels of 10^3-10^5 organisms per gram. The sausage was fermented at 30°C for 18-24 hours after inoculation. There

was a further decrease in S. typhimurium upon storage at 5°C. However, the organism still was detectable in vacuum packaged, sliced thuringer sausage after 42 days storage. Smith et al. (119) determined that S. dublin and S. typhimurium, when inoculated at levels of 10^4 organisms per gram into Lebanon bologna (final pH of 4.3-4.4), died by the end of the fermentation period. It would appear that salmonellae do not survive well in low pH sausage.

Possibly because there are few reports of the incidence of enteropathogenic E. coli in foods, E. coli are rarely considered classical food poisoning organisms (26,52). Surkiewicz et al. (126) found E. coli in 1 of 690 packages of frankfurters. Hughes (68) reported E. coli levels of 10^3 organisms per gram in vacuum packaged, cooked meat which had been exposed to abusive storage conditions.

Similarily, there are few reports of B. cereus in cooked, cured meats. This lack of information probably is due to the organism not being given much attention. However, B. cereus frequently is found in the environment and in foods. Goepfert et al. (47) stated that 52.5% of 431 meat and vegetable products and 7.7% of sausage products contained B. cereus. Graham and Blumer (50) reported that Bacillus was the most common genus observed in preserved meat.

C. perfringens, like B. cereus, is distributed widely

in nature. Its incidence in cooked, cured meat is less significant. Cooked, cured meats rarely have been implicated as a vehicle of C. perfringens food poisoning (71,105, 106,107). Clostridium spp., however, have been isolated from sandwiches and vacuum packaged meats. Insalata et al. (72) reported that 1 of 400 samples of frankfurters contained spores of C. botulinum type B. C. botulinum type B toxin was detected in 1 of 73 samples of vacuum packaged luncheon meat (cited in 72). Christiansen and King (22) observed C. perfringens, at a level detectable by enrichment, in 1 of 62 samples of prewrapped sandwiches. Another survey, of 108 prewrapped sandwiches, revealed C. perfringens in 9.2% of the samples (cited in 22).

C. Indicator Organisms

Classical pathogens (e.g. S. aureus, Salmonella spp., C. perfringens) do not represent the only pathogens which may be present in foods. Viruses and other less frequently considered bacterial pathogens also may contaminate foods. Routine analysis for all possible pathogens in foods represents an impossible task. Analysis for indicator organisms is quicker and less expensive, and provides much of the information required to evaluate a food's safety.

Because most food-borne pathogens are associated with mammalian feces, fecal indicators usually are analyzed. The ideal fecal indicator should occur only in the intestine and

in high numbers in the feces. Furthermore, it should be resistant to the extraenteral environment, and should permit easy and reliable detection (17). While an ideal organism does not exist, E. coli, Lancefield Group D streptococci, and clostridia are considered to be good indicators (17). E. coli is most numerous in feces, but it is least resistant to the extraenteral environment (17). Group D streptococci are less numerous but more resistant than E. coli (17,134). Of the Group D streptococci only S. faecalis, S. faecium and S. bovis are associated with feces. S. durans is not of fecal origin but is often observed in heat processed meats (124).

Coliforms, fecal coliforms and E. coli have been used traditionally as indicators of fecal contamination of food and water (1,17,60,134). Group D streptococci have been used to a lesser extent (41,52,93,99,134). Correlations between these two types of indicators are poor (17,134). Clostridia have seldom been used as indicators of fecal contamination (17).

Enumeration of E. coli is lengthy, and not always an accurate procedure (17,124,134). Buttiaux and Mossel (17) recommended that Enterobacteriaceae, enumerated on MacConkey's VRBA with 1% D-mannitol, and Group D streptococci be used together to provide more reliable results than E. coli alone.

When interpreting results for indicator organisms, it

is important to remember that fecal pollution of food usually is small at the point of contamination. Direct fecal contamination is claimed to result in the transfer of less than 100 Enterobacteriaceae and less than 10 Group D streptococci per gram when the rate of fecal transfer is 25 mg per 10 kg of food (17). High counts of indicator organisms usually do not indicate high levels of fecal contamination, but rather indicate bacterial multiplication during storage and/or preparation (17,134). High counts of indicator organisms, therefore, suggest exposure to conditions which might introduce pathogens and might allow growth of pathogens (134).

The total aerobic mesophilic count is used occasionally as an indicator count in non-fermented foods (134). This count may indicate contaminated raw materials, unsatisfactory sanitation, inadequate heat processing, and/or abusive storage (134). However, total aerobic count should not be used as indicator counts with vacuum packaged foods, cheese or refrigerated meats (11,58). Unfortunately, these counts sometimes are misused or misinterpreted: Hughes (68), in describing the case of a retailer in England charged with selling vacuum packaged luncheon meats considered unfit for human consumption, supported the charge with laboratory results indicating a total count of $1\text{-}3 \times 10^7$ organisms per gram.

3. Factors Affecting the Survival and Growth of Pathogens in Bologna and Related Products

Cured meats present a complex micro-environment for surviving and contaminating microorganisms. This environment, in addition to certain processing procedures, functions in selecting the microflora, and affects survival and growth of pathogens. The more generally recognized inhibitory and selective substances and procedures relevant to bologna will be discussed.

A. Inhibitory and Selective Constituents

(i) Nitrite

Nitrite has probably been used in curing meats for centuries. In Canada, it is permitted in preserved meats and preserved meat by-products, except side bacon, at levels of 200 ppm (rate of addition) (38). In cured meats nitrite serves several important functions. Nitrite, added as sodium nitrite, through decomposition products, reacts with the meat pigments (i.e. myoglobin and hemoglobin) to form nitrosomyoglobin (-hemoglobin). Application of heat and smoke converts nitrosomyoglobin to the stable pink pigment (i.e. nitrosohemochrome) characteristic of cured meats (4,71,81). A nitrite concentration of 20 ppm is required for color fixation (71).

The second function of nitrite is also organoleptic.

Nitrite, added at levels of 50 ppm, contributes to the characteristic flavor of cured meats (71,81,116).

Non-organoleptic functions of nitrite are inhibition of food poisoning and food spoilage bacteria, and protection against development of rancidity (71,81,116). A concentration of 100 ppm of nitrite is required to prevent germination of C. botulinum spores (71). However, the actual level of nitrite required for effective antimicrobial activity is dependent upon pH, salt concentration, temperature, oxygen partial pressure, and other factors (34,49,71,75,81,106,108). Ingram (71) reported that pH 5.5 was optimal for antimicrobial activity and that nitrite was less effective aerobically than anaerobically.

The mechanism of nitrite's antimicrobial activity is not completely understood. The American Meat Institute Foundation (4) suggested that the bacteriostatic effect on S. aureus may have been due to interference with sulfur nutrition. Ingram (71) stated that nitrite inhibited bacteria by acting as a general metabolic inhibitor. Riha and Solberg (106) suggested that nitrite, in some form, inhibited growth by reacting with enzymes containing functional sulfhydryl groups. Buchanan and Solberg (15) suggested that nitrite blocked the sulfhydryl sites of either coenzyme A or alpha-lipoic acid and thus blocked normal pyruvate metabolism.

The level of nitrite in cured meats does not remain constant; it decreases during heat processing and with time. Sebranek et al. (115) reported a 60% drop in the nitrite added to comminuted meat (156 ppm) during heat processing. During subsequent storage at 5°C the nitrite level dropped, at a decreasing rate, to a final constant level of 20% of the original concentration.

The rate of nitrite loss during storage is a function of pH and temperature (4,71,101). The depletion rate increases as the pH of the product drops (4,71,101). In an acid environment a relatively large percentage of nitrite is present as nitrous acid (in comparison to a non-acid environment). Nitrous acid is highly reactive, and reacts with organic matter (e.g. amino groups) (71,81). The effect of pH on depletion rate decreases as the concentration of nitrite decreases (101). Storage temperature has a direct effect on the logarithm of the nitrite half-life (71,101).

Depletion of nitrite suggests that nitrite cannot be depended upon as a preservative in processed and stored meats (4). Ingram (71), however, stated that antimicrobial activity was not necessarily decreased as nitrite disappeared. Nitrite might be converted into other inhibitory substances. Riha and Solberg (106,107) supported this view, reporting that heating a medium containing nitrite produced an inhibitor more potent than nitrite

itself.

(ii) Acidity and pH

Development of acidity in bologna appears to be variable. Reimann et al. (105) reported that pH of commercial bologna (6 samples) was 5.2-5.3. Steinke and Foster (122) observed that pH of bologna remained in the range of 6.2-6.4 during 15 weeks of storage at 5° and 10.5°C. Kempton and Bobier (77) reported pH 5.0 in bologna after 15 weeks storage, but noted that pH did not begin to drop from its initial value of more than 6.5 until after 4 weeks of storage. Decrease in pH was mirrored by accumulation of lactic acid.

Acid production in cured meats is a function of the nature of contaminating microflora (29,44,69,70,105,106) and of concentration of fermentable carbohydrate (105). Lactic acid bacteria (lactobacilli, streptococci, leuconostocs, and pediococci) are the organisms primarily responsible for acid production (3,77,93,118,139). These organisms are heat sensitive and are destroyed during processing (77). The variable nature of contamination during slicing and processing may account for the different pH levels observed in bologna (70).

The preserving action of low pH and organic acid concentration may be the result of synergism (28,70,105,106)

or direct toxicity of organic acid (29). A low pH may provide an environment in which a metabolite of lactic acid bacteria exerts maximum inhibition (29). Riemann et al. (105) reported that the combination of NaCl, anaerobiosis, and refrigerated storage narrowed the pH range for growth and toxin production by S. aureus. Ingram (71) suggested that optimum antimicrobial activity of nitrite was achieved at pH 5.5. Riha and Solberg (106) reported that lower concentrations of nitrite were required for inhibition of C. perfringens at lower pH. Dack and Lippitz (28) stated that pH was only partially responsible for inhibition of S. aureus, S. typhimurium and E. coli in pot pies. Jensen (75) stated that antimicrobial activity at constant pH was dependent on the nature of the acid molecule. The order of effectiveness was: acetic acid > citric acid > lactic acid >> HCl. Minor and Marth (92), on the other hand, reported the order of effectiveness against S. aureus was acetic > lactic > phosphoric > citric = HCl. These observations suggest that undissociated acid molecules rather than simply the hydrogen ion concentration are involved in inhibition (91). Inherent cell physiology may determine which organisms are most inhibited and which acids are most inhibitory (91).

(iii) Salt (NaCl) Concentration and Available Water

Salt is the only ingredient essential in meat curing (4,82). The major function of salt is to inhibit microbial

growth by decreasing the available water (Aw) of the product (82). In emulsion-type sausage, salt also serves to solubilize the myosin from the muscle fibre (4).

Food-borne pathogens can grow aerobically in the Aw range of 0.83-0.999 (70,137). The optimum Aw is 0.98 or greater (4). The American Meat Institute Foundation reported that the Aw of 3.5% Brine was 0.98, of 7% Brine was 0.96%, of 10% Brine was 0.94, and of 16% Brine was 0.90. Ingram (70), however, reported that 12% and 15% Brine were equivalent to Aw's of 0.90 and 0.86, respectively. Ingram (70) also reported that the Aw of cured meats was 0.90-0.95. Riemann et al. (105), however, reported that the brine concentration of commercial bologna (6 samples) was only 3.0-5.3%.

These salt concentrations will permit growth of certain pathogens [e.g. S. aureus and B. cereus (137)]. Lactic acid bacteria and micrococci also should multiply in cured meats (2,102,122). Organisms which develop in environments with low Aw experience increased lag times, increased generation times, and decreased maximum stationary populations (34,137).

NaCl inhibitory activity increases in combination with other factors. Troller (137) reported that enterotoxin B production by S. aureus was dependent upon interaction of pH and NaCl concentration. Ingram (71) and the American Meat

Institute Foundation (4) reported that bacterial inhibition was dependent upon the interaction of pH, nitrite, NaCl concentration, temperature and oxygen partial pressure. Riemann et al. (105) reported that NaCl alone, used at commercially acceptable levels, could not prevent S. aureus growth and enterotoxin production. Dempster (34) stated that salt was less effective at lower storage temperatures because the velocity of the bactericidal reaction was decreased.

(iv) Competitive Growth

References concerning the effects of competitive growth on pathogens in vacuum packaged cured meats are limited. Bartl (11) stated that enterococci were antagonistic to Clostridium and Bacillus spp. in canned hams. In other products, it appears that pathogens do not compete well with the natural flora. Dack and Lippitz (28) reported that the natural flora in pot pies stored at 35°C inhibited growth of S. aureus, S. typhimurium and E. coli even when pathogens outnumbered the natural flora. Christiansen and King (22) observed only slight increases in S. aureus inoculated into sandwiches at 10^2 organisms per gram after 48 hours at 37°C. S. aureus appeared to be overgrown by the sandwich flora.

The mechanism whereby one organism inhibits another can take a variety of forms. Inhibition may involve antibiotic production (e.g. nisin production by S. lactis), hydrogen

peroxide production (e.g. clostridia and staphylococci are sensitive to hydrogen peroxide produced by L. lactis and L. bulgaricus), nutrient depletion (e.g. micrococci deplete amino acids which are essential to S. aureus growth), decrease in oxidation-reduction potential (e.g. staphylococci inhibition of micrococci), or organic acid production and pH alteration (29,30,44,69). Nutrient depletion probably is not involved when considering inhibition by lactic acid bacteria (44). Inhibition occurs too rapidly for this mechanism to be involved. Also, lactic acid bacteria are nutritionally more fastidious than most pathogens. Acid production and pH alteration appears to be the primary mechanism of S. aureus, Salmonella and C. perfringens inhibition by S. diacetilactis (29).

(v) Oxygen and Carbon Dioxide Partial Pressure

Using commercial vacuum packaging equipment, an oxygen partial pressure of at least a few mm of mercury remains in vacuum packaged meats (79). This residual oxygen is consumed by meat tissue and bacterial respiration (4,79). At the same time carbon dioxide is liberated and acts as a selective inhibitor (70). Partial pressures of oxygen and carbon dioxide play a major role in the selection and development of the microflora (3,70). Ingram (70) and Alm (3) reported that lactobacilli and Lactobacteriaceae growth in general was selected by vacuum packaging. Davidson et al. (31)

suggested that the depletion of oxygen and the increase in carbon dioxide selected M. thermosphactum. Sutherland et al. (127), however, reported that M. thermosphactum comprised only 5% of the flora of vacuum packaged fresh beef.

In addition to having an effect on the composition of the microflora, vacuum packaging also affects the maximum stationary population. The maximum population in vacuum packaged meats (fresh and cured) rarely exceeds 10^8 organisms per gram (2,70,77,110,139), whereas the maximum count in aerobic packages often exceeds $1x10^9$ organisms per gram (70,110). Christiansen and Foster (21) found that vacuum packaging inhibited S. aureus growth on sliced ham.

Synergistic effects may again be involved when selection and inhibition by oxygen partial pressure and carbon dioxide partial pressure are considered. Roth and Clark (110) suggested that other factors were involved because fresh meat spoilage organisms developed at 20% carbon dioxide and 0.5% oxygen. Ingram (71) reported that nitrite was more effective in the absence of oxygen. Riemann et al. (105) stated that S. aureus was inhibited by a combination of NaCl, anaerobiosis, refrigerated storage and low pH.

(vi) Combinations of Inhibitory Factors

Much bacterial inhibition observed in cooked, cured

meats is due to combinations of factors. These combinations may result in synergistic or simply in additive inhibition. The examples of specific inhibitor combinations which are cited in the previous sections illustrate the errors of interpretation which may occur if inhibitors are considered separately.

B. Procedures Affecting Survival of Pathogens

(i) Processing Conditions

The processing conditions which affect microbiological quality of cooked, cured meats are smoking, cooking and slicing.

The primary functions of smoking are development of flavor, preservation, development of color, and protection against oxidation (82). Flavor effects result from the incorporation of smoke components (e.g. phenols and carbonyls) into the product. Smoke functions in color development through roles in nitrosohemochrome formation and non-enzymatic browning. The preservative activity of smoke is due to bacteriostatic and bactericidal properties of smoke components (e.g. phenols and organic acids) and to removal of moisture from the product surface. Antioxidant activity of smoke probably is due to phenols preventing oxidative changes.

Cooking reduces microbial numbers (82), sets the

emulsion by coagulating and denaturing the protein, functions in nitrosohemochrome formation, and inactivates proteolytic enzymes. The preservative activity of cooking results from heat destruction of bacteria and from surface drying of the product. Heat destruction of bacteria is a function of time and temperature of cooking (5,82) and of production of inhibitory substances (106,107). Commercial heat processing at 60°C (140°F) or above destroys S. aureus and Salmonella spp. (4). Spores are not affected by commercial heat processing.

Slicing, unlike smoking and cooking, has no direct effect on the survival of pathogens. Slicing is responsible for most of the post heat processing contamination (77) and, therefore, for determining the nature of the microflora of cooked, cured meats (70).

(ii) Storage Temperature

Most pathogens are mesophiles. Mesophiles have optimum growth temperatures of 35° to 40°C and minimum growth temperatures of 10° to 15°C (4,121). However, certain mesophiles, including pathogens grow slowly at 5° to 10°C (5,128). Refrigerated storage should inhibit mesophile growth, but permit growth of psychrophiles. Holtzapffel and Mossel (67) reported that lactobacilli developed as the primary flora in salads stored at 9°C. Bartl (11) reported that lactic acid bacteria developed in cured meat stored

below 10°C. Refrigerated storage also affects the rate of microbial growth. Allen and Foster (2) stated that microbial growth in bologna was slower (i.e. increased lag phase, increased generation time and decreased maximum stationary population) at 1.1°C vs 7.2°C.

Temperature of storage not only has a direct effect on the growth of pathogens, but also has a synergistic effect in combination with other inhibitory factors. Interaction of temperature, pH, Aw, oxygen partial pressure, carbon dioxide partial pressure, competitive growth and nitrite concentration is most responsible for bacterial inhibition and selection (4,34,71,77).

4. Characteristics of Pathogens and Mechanisms of Pathogenicity

A. Staphylococcus aureus

S. aureus is a facultative anaerobe which is ubiquitously distributed in man's environment. As many as 30-50% of normal adults are nasal carriers (7). The organism is mesophilic, salt tolerant, and non-spore forming. S. aureus food poisoning is due to enterotoxin production in food prior to ingestion. Growth of S. aureus and enterotoxin production in foods is affected by inhibitory substances, competition and storage temperatures (42,128).

Buchanan and Solberg (15) reported that 200 ppm nitrite

inhibited S. aureus in vacuum packaged cured meats. Minor and Marth (91), on the other hand, reported that 200 ppm nitrite was an ineffective inhibitor of growth and enterotoxin production. Differences in pH and/or oxygen partial pressure may account for these apparent contradictions.

S. aureus grows in the pH range 4-10 and produces enterotoxin in the range 4-9.8 (128). Jensen (75) reported that the lag phase was prolonged at pH 5.8 and below. The minimum pH for growth and enterotoxin production in food depends on a number of factors. The minimum pH was 5.0 when lactic acid was the acidulant (122), 5.0-5.1 in aerobically packaged cured meats (10,105,114), 5.7 in anaerobically packaged fermented sausage (10), 5.6 in cured meat at 10°C and 5.3 in cured meat at 30°C (42).

The optimum Aw for S. aureus growth and enterotoxin production is 0.99 (70,128,137). S. aureus was inhibited by a brine concentration of 10% in vacuum packaged cured meat (105). The Aw of cured meat varies from 0.90-0.95 (70). As the Aw drops from the optimum, S. aureus experiences increased lag time, decreased exponential growth rate, and decreased maximum stationary population (76,137,138). Enterotoxin A production is affected less by decreases in Aw than is enterotoxin B production (91). The level at which Aw limits growth is affected by other inhibitory factors. The

minimum Aw for growth under aerobic and anaerobic conditions is 0.86 and 0.90, respectively (70). Heat stressed S. aureus are sensitive to 4% NaCl (120).

S. aureus growth and enterotoxin production in mixed cultures is limited (28,30,36,44,90,113,128). Lactic acid bacteria are especially effective inhibitors (28,30,44). Growth may not result in enterotoxin production in a competitive environment (113,128).

Tatini (128) reported growth of S. aureus between 7° and 47.8°C, enterotoxin production between 10° and 46°C, optimum growth at 37°C, and optimum enterotoxin production between 40 and 45°C. Enterotoxin B production was observed in cured meat stored at 10°C for 16 weeks (42,105) and at 22° and 30°C for 2 and 3 days (42). Enterotoxin A production was observed between 13° and 45°C (114). Temperature limits for growth and enterotoxin production are affected by other inhibitory factors, discussed previously.

There are 6 known, etiologically distinct, enterotoxins (A,B,C,D,E and F) produced by S. aureus (104). Enterotoxin A is involved in most staphylococcal food poisoning (10,91,104,113,134), whereas enterotoxin B is the least frequently involved toxin (104). Despite this, enterotoxin B is used most often when evaluating environmental and processing condition effects, probably because it is produced most easily in the laboratory (104). Unfortunately,

enterotoxins A and B differ in their response to many conditions. Enterotoxin A is less heat tolerant and less sensitive to low pH than enterotoxin B (91). Enterotoxin A is produced at lower Aw than enterotoxin B (91). These differences may be functions of the formation of the enterotoxins (91). Enterotoxin A is a primary metabolite (secreted mainly during exponential growth), whereas enterotoxin B is a secondary metabolite (secreted during the stationary phase).

B. *Salmonella* spp.

Salmonella food poisoning is an infection caused by the ingestion of living organisms which multiply within the small intestine (130). The infective dose varies with the virulence of the serotype (134). Angelotti *et al.* (5) reported that oral infective doses for healthy adults varied from 5.87×10^5 for *S. melagarides* to 6.72×10^7 for *S. anatum*. Certain serotypes (e.g. *S. pullorum* and *S. gallinarum*) rarely are implicated in human salmonellosis (134). The survival and growth of salmonella in food is affected by processing conditions, inhibitory substances, competition and storage temperature (33).

Angelotti *et al.* (5) reported a 6D reduction in *S. senftenberg* 775W heated to 65.5°C for 12 minutes and a 4D reduction at 60°C for 45 minutes in several multiple foods. *S. senftenberg* 775W is a heat resistant salmonella (5,102).

Cooking of cured meats should affect total salmonella destruction (33,119).

Salmonellae should not be inhibited by commercial levels of nitrite alone. Davidson and Webb (33) reported that the minimum inhibitory concentration of nitrite at pH 6.7 was 800-4,000 ppm. Goepfert and Chung (45) found that S. typhimurium was able to grow in the presence of both 100 and 200 ppm nitrite at pH levels of 5.0 and 5.5

Under conditions that would otherwise be considered ideal, salmonellae were able to grow at pH 4.05 when HCl was the acidulant (23). When lactic or acetic acid was used, the minimum pH was 4.40 and 5.40, respectively (23). In cheddar cheese, a pH of 5.7 or lower contributed to salmonellae decline during aging (103,141). Fatty material, especially low molecular weight fatty acids, inhibited salmonellae in meat and bone meal (78). S. typhimurium multiplied in low acid (pH 5.2-5.4) sausage during fermentation at 30°C, but not during fermentation of regular acid (pH 4.8-5.1) product (23). The combination of inhibitory factors increased generation times in the low acid sausage. Smith et al. (119) also observed death of salmonellae during regular sausage fermentation.

Brine concentrations of 8% NaCl (33,45) or an Aw of 0.94 (33) are inhibitory to salmonellae. At Aw less than 0.99, salmonellae experience increased lag phase and

decreased maximum stationary population (137). Combinations of inhibitors affect salt tolerance. Matches and Liston (85) reported growth of S. typhimurium in 0-1% NaCl at 8°C, in 0-4% NaCl at 12°C, and in 0-8% NaCl at 22° and 37°C.

Salmonellae are facultative anaerobes (33). As with S. aureus, survival and growth decreases in anaerobic vs aerobic environments (45). Competition, especially from acid producing bacteria, reduces the growth rate of salmonellae (28,29,44,46,103). Gilliland and Speck (44) observed antagonism of S. gallinarum by lactic streptococci even at constant pH. Antagonism increased when pH was allowed to drop.

Salmonellae do not grow below 5°C (5) and, in fact, may die during storage in certain products (e.g. thuringer sausage) at 5°C (45). Greatest tolerance to low pH is exhibited at 25° to 32°C (23).

Most of the salmonella inhibitors cited above are at levels in excess of those found in cooked, cured meats. Inhibition, therefore, is dependent upon satisfactory refrigeration and interaction of inhibitory factors (23,33,119). Unfortunately, satisfactory refrigeration is not always maintained during distribution and handling (13,33).

C. Enteropathogenic *Escherichia coli*

E. coli is a lactose fermenting organism of the family *Enterobacteriaceae*. In foods, *E. coli* usually is considered an indicator organism (134). Only recently has consideration been given to the pathogenicity of *E. coli* in situations other than infantile diarrhea (14,26,134). Enteropathogenic *E. coli* can be divided into 2 groups according to the symptoms (10,60). The first is an invasive *Shigella*-like group which produce a dysentery-like syndrome as a result of invasion of, and multiplication in, the intestinal mucosa. Enterotoxin may not be involved. The second type is a non-invasive *Salmonella*-like group which produce gastroenteritis as a result of multiplication in the intestine. Enterotoxin may function in the diarrheal syndrome. Non-invasive enteropathogenic *E. coli* can elaborate either heat-labile or heat-stable enterotoxin (48). Heat-labile toxin producing strains are thought to be of major importance in "travellers' diarrhea" in Mexico (48). Finklestein *et al.* (37) reported that ability to produce enterotoxin is genetically determined by plasmids or by transmissible extrachromosomal material and suggested that any strain of *E. coli* may, therefore, become enterotoxigenic.

Most enteropathogenic *E. coli* are of the non-invasive type (111). Large numbers (not specified) of non-invasive enteropathogenic *E. coli* must be ingested before illness

occurs (112). Multiplication must occur in the food prior to ingestion. Effects of inhibitors associated with cooked, cured meats on enteropathogenic E. coli are not cited in the literature reviewed.

D. Bacillus cereus

B. cereus is an aerobic, spore-forming organism often found in soil, on vegetation and in foods (134). This organism has only recently been given serious consideration as a food poisoning organism in North America (47). The mechanism of pathogencity is not clear (47,48). It appears to be an intoxication similar to that caused by C. perfringens except that the enterotoxin, produced during exponential growth, is released without cell lysis (48). The rapid onset of symptoms [8-16 hours (89)] suggests that toxin is produced in food prior to ingestion (47,48,54). Therefore, multiplication would have to occur in food prior to ingestion. Levels of 10^6 or more organisms per gram of food are required to produce illness (54,134).

The effects of inhibitors associated with cured meats on B. cereus have not been evaluated thoroughly. Basic growth characteristics of the organism are cited in review articles by Goepfert et. al (47) and Troller (137). B. cereus has a growth range of 10°-50°C with optimum growth at 30°C. On laboratory media, adjusted with mineral acids, growth will occur above pH of 4.9. B. cereus tolerates 5-7%,

but not 10% NaCl. The normal saprophytic flora in most foods inhibit growth of B. cereus. Group D streptococci, in particular, appear to antagonize B. cereus (47). Because it is a spore-former, B. cereus may survive heat processing of cooked, cured meats. Graham and Blumer (50) reported Bacillus spp. survived pasteurization (80°C for 10 minutes) in Trypticase Soy Broth.

E. Clostridium perfringens

C. perfringens is an anaerobic, spore-forming organism commonly found in dust, water, mammalian feces and in many foods (6,64). The spores have D values up to 1 hour at 100°C (134). The temperature range for growth is 7°-50°C (optimum 43°-47°C) (64). The organism is inhibited at or below pH 6.1 (64), by 5-6% NaCl (64,137), by 400 ppm nitrite (137), by hydrogen peroxide produced by competing microflora (29,69), by refrigerated storage (64), by aerobic environments, or by combinations of these factors. Hobbs (64) reported that growth was inhibited by 25 ppm nitrite plus 5.3% NaCl. Heat increases inhibition in nitrite-containing media (106,107). Lower levels of nitrite inhibit C. perfringens at pH 6.3 than at pH 7.2 (106).

C. perfringens food poisoning is caused by growth, sporulation and subsequent cell lysis releasing enterotoxin in the enteric system (106). Populations of 10^6 organisms/gram of food are required to produce illness

(134). This again suggests that there must be considerable multiplication in the food prior to ingestion.

F. Other Pathogens

In addition to the pathogens already discussed, a variety of organisms, normally not considered to be pathogenic, may cause food poisoning when consumed in large numbers (134). Thatcher and Clark (134) stated that fecal streptococci, Proteus and pseudomonads have been suspected as agents of food poisoning. Of these organisms, the fecal streptococci (i.e. S. faecalis, S. faecium and S. bovis) have received the most attention (4,12,94).

Implication of fecal streptococci in food poisoning usually is based upon isolation of large numbers (10^6-10^8/g) of these organisms in foods apparently involved in a food poisoning outbreak (12,94,134). Moore (94) reported that some human volunteers were susceptible to fecal streptococci poisoning in feeding studies, but that not all strains were toxigenic.

Fecal streptococci are frequently isolated in large numbers in a variety of foods (12,94,134). If these organisms are pathogenic it is surprising that the incidence of confirmed fecal streptococci food poisoning is so very low (12,94). Moore (94) suggested that this may be due either to failure of the public to notify health authorities

of mild illness or the result of other conditions necessary for illness not being present.

5. Epidemiological Information

In Canada there were 69 incidents (1,516 cases) of microbiological food poisoning reported in 1973 (135). Of these, 33 incidents (600 cases) were attributed to S. aureus, 14 incidents (334 cases) to Salmonella, 7 incidents (535 cases) to C. perfringens, 3 incidents (19 cases) to B. cereus, and 2 incidents (4 cases) to streptococci. There were an additional 289 incidents (1,733 cases) of food poisoning where the etiological agent was not identified. Meat products were vehicles in 31% of incidents. Sausages were associated with 26 incidents (66 cases).

The United States Center for Disease Control reported 1,703 outbreaks (97,590 cases) of food poisoning in the U.S.A. between 1968-72 (cited in 14). S. aureus, Salmonella and C. perfringens were involved most frequently. Duncan (35) reported that C. perfringens accounted for 28% of the outbreaks (49% of the cases) in the U.S.A. in 1968. Beef and poultry were the most common vehicles. Angelotti (7) reported that S. aureus, implicated in 250 outbreaks (10,000 cases) in 1952-61, was the most important food poisoning agent in the U.S.A.

Hobbs (65) reported an average of 6,000 outbreaks of

food poisoning per year in England and Wales between 1968-70. Salmonellae were responsible for the majority of outbreaks. C. perfringens and S. aureus were occasionally involved. Meat and poultry were vehicles in 80-90% of the outbreaks. Processed meat and poultry were vehicles in 10-12% of the outbreaks. In 1966, S. typhimurium was responsible for 1,407 outbreaks (2,346 cases) of food poisoning in England and Wales (64). C. perfringens was responsible for 63 outbreaks (1,947 cases), and S. aureus caused 54 outbreaks (262 cases). Angelotti (7) cited that 92-95% of food poisonings in the U.K. and Wales were due to Salmonella, but only 5% or less were due to S. aureus in 1961-63.

Goepfert et al. (47) reported that B. cereus was ranked third as the causative agent of food poisoning in Hungary, accounting for 8.8% of the outbreaks (15.2% of the cases) between 1960-68. Meats, including sausage, and high carbohydrate dishes (sauces) which were exposed to temperature abuse were the most common vehicles. Angelotti (7) noted that there were numerous cases of B. cereus food poisoning in the Netherlands.

Enteropathogenic E. coli has not received sufficient attention to suggest its incidence as an agent in food poisoning (20,129). Gorbach et al. (48) determined that 72% of "travellers diarrhea" in Mexico was caused by

enterotoxigenic E. coli. Reported vehicles of enteropathogenic E. coli food poisoning included coffee substitute, red bean balls, stewed meat and gravy, roast mutton, pork and chicken (cited in 134).

Sandwiches rarely were reported as vehicles of food poisoning (1,87). A review of Morbidity and Mortality Weekly Reports (95) for the years 1970, 1972-73 revealed only 4 outbreaks associated with sandwiches. Other reports supported these findings (1,87). Between 1951-63, 133 incidents (5,947 cases) were attributed to sandwiches in the U.S.A. (cited in 87). In almost every case however, the sandwich filling was unfit for consumption before it was used in the sandwich. In contrast, Todd (135) reported a slightly higher incidence. In Canada, in 1973, 18 (4.8%) incidents (173 cases) were sandwich associated. Egg salad sandwiches contaminated with S. aureus were responsible for the largest outbreak (125 cases).

Cured meats rarely have been implicated in C. perfringens (105,106,107) or salmonella (33,105) food poisoning. However, cooked, cured meats eaten cold have been frequent vehicles of S. aureus food poisoning (64). References indicating the incidence of B. cereus and enteropathogenic E. coli in cooked cured meats were not available.

Chapter III. Materials and Methods

1. Bologna Survey

A. Sample Specifications

Vacuum packaged, sliced bologna, labelled "Bologna", was the only variety of bologna that was sampled.

B. Sampling Plan

Bologna of two age categories, from three types of retail display and produced by six different manufacturers was sampled. The age categories considered were "new" (i.e. less than 15 days shelf-life expired) and "old" (i.e. more than 21 days of shelf-life expired). The three types of retail display were "pegboard", "open chest", and "open case section of a pegboard". In addition, samples of new bologna were obtained at the retail level and stored in the laboratory at 4°C, until expiry date. A minimum of five different retail stores were sampled for each of the "pegboard" and "open chest" display types. "Pegboard" samples were obtained from at least one of each of the major Edmonton grocery retail chains (i.e. Canada Safeway Ltd., IGA Food Supermarkets, Woodwards Stores Ltd. and Edmonton Co-op). The manufacturers sampled were Burns Meats Limited (Establishment 1A), Inter-Continental Packers (Establishment 69), Gainers Limited (Establishment 27), Swift Canadian Co. Ltd. (Establishment 18B), Canada Packers Ltd. (Establishment

7A), and J.M. Schneider Inc. (Establishment 35). Retail store "House Brands", in addition to these manufacturers' brands were also sampled.

C. Sample Collection

All samples, except those intended for "laboratory" storage, were obtained from the retail stores 1 to 3 days prior to analysis. Only 6 oz., 8 oz., or 12 oz. packages were sampled. Following collection, all samples were stored at 4°C until they were analyzed. Only "new" bologna was obtained from retail stores for "laboratory" storage.

D. Sample Preparation

An 11 g wedge of bologna was aseptically cut through all slices and weighed. This wedge was used for all bacteriological determinations, except salmonellae, and was homogenized with 99 ml of sterile, 0.1% peptone water in a Waring Blendor at high speed for 2 min.

For salmonella detection, a 25 g wedge was homogenized with 150 ml sterile nutrient broth (Difco).

E. Sample Analysis

Appropriate dilutions of the homogenized 11 g samples were plated in duplicate onto the following media. All media were Difco brand (Difco Laboratories Inc. Detroit, Michigan), unless otherwise stated.

Total counts (SPC) at 4°, 21° and 36°C were determined on Plate Count agar. The plates were incubated for 10 days, 72 hours and 48 hours, respectively.

Lancefield's Group D streptococci were determined on KF Streptococcus agar incubated at 36°C for 48 and 72 hours (51).

Lactic acid bacteria were determined on nitrite-actidione-polymyxin (NAP) agar prepared as directed by Davidson and Cronin (32) and incubated at 30°C for 72 hours. Randomly picked colonies from NAP agar were screened using a Gram stain and catalase test (41).

Lactobacilli were determined on Rogosa et al. (109) LBS agar, adjusted to a pH of 5.60 ± 0.05 according to Costilow et al. (25), and incubated at 30°C for 72 and 120 hours.

Microbacterium thermosphactum were determined on STAA agar prepared according to Gardner (40) and incubated at 21°C for 72 hours. Countable STAA plates were flooded with 2-3 ml of oxidase reagent (N,N-Dimethyl-p-phenylene-diamine Monohydrochloride-Eastman). Oxidase positive colonies were excluded from the count on STAA.

Clostridia were determined on tryptose-sulfite-cycloserine (TSC) agar (53) and egg-yolk free TSC agar (56). TSC and egg-yolk free TSC were incubated anaerobically in a

hydrogen/carbon dioxide atmosphere (using BBL "gas-paks") at 36°C for 24 hours.

Staphylococci were determined on mannitol salt (MSA) agar, and on Baird-Parker (BP) medium. Both media were incubated at 36°C for 48 hours. Colonies on BP were counted according to Health Protection Branch procedures (59), and confirmed by streaking on MSA slants and by the coagulase test (59) using EDTA coagulase plasma.

Coliforms and fecal coliforms were determined using the 3-tube most probable number (MPN) technique adapted from the Health Protection Branch (60) and Thatcher and Clark (134). Lauryl Tryptose (LST) broth was inoculated with appropriate dilutions of bologna homogenate. Gas positive LST tubes at 24 and 48 hours were inoculated into Brilliant Green Lactose 2% Bile (BGB) broth and into EC medium. Gas positive BGB tubes at 24 and 48 hours were streaked onto Levine's EMB agar for the completed coliform test. All media, except EC medium, were incubated at 36°C. EC medium was incubated at 44.5 ± 0.05°C for 24 and 48 hours. Gas positive EC tubes provided the MPN for fecal coliforms.

Salmonellae were determined by homogenizing 25 g of product in 150 ml of nutrient broth (non-selective enrichment) adjusted between pH 6.0 and 7.0 (61). After incubation at 36°C for 20 hours, an aliquot of homogenate was transferred to selenite-cystine broth (selective

enrichment). Brilliant green (BGA) and bismuth-sulfite (BSA) agars were used as selective plating and purification media. Salmonella-type colonies on BGA and BSA were screened on MacConkey agar and TSI slants. All media were incubated at 36°C for 24 and 48 hours.

Positive and negative media controls were run periodically throughout the survey. Known cultures of S. aureus, S. typhimurium, S. cholerae-suis, and C. perfringens were used with the positive controls.

In addition to the bacteriological tests, pH of the bologna samples was measured. Two procedures were used in this study. A Fisher Accumet (Model 230) pH meter with glass and reference electrodes was used to determine the pH of a 1:10 dilution of bologna (homogenized) in de-ionized, distilled water (77,139). Also, for ca. 70% of the samples, the pH was determined by inserting a combination (single probe) electrode (Fischer Scientific Co.) directly between the slices of the bologna (110,113,114,118).

2. Inoculation Study

A. Sample Specifications

Freshly sliced, but not packaged, bologna was obtained directly from two manufacturers. The manufacturers were chosen on the basis of the bologna survey results which indicated that high pH (i.e. pH near 6.5) and low pH (i.e.

pH below 5.5) bologna could be obtained by sampling product from these firms. All samples were manufactured 3-5 days prior to slicing and packaging.

B. Variable Evaluation

The sampling procedure was designed to determine the effect of bologna age and pH, and of incubation time and temperature, on the growth of several pathogens. Triplicate measurements were obtained for each variable. Bologna age was evaluated by analyzing high pH bologna after 2 and 30 days of storage. The 2 day old product represented fresh product, while 30 day old samples represented product near the expiry date. The effects of bologna pH were evaluated using old bologna obtained from the two manufacturers discussed. These manufacturers were chosen on the basis of the survey results mentioned above. All samples were incubated at 4°, 21° and 30°C for 0, 4, 8 and 25 hours. The samples were inoculated with *S. aureus* S-6, *S. typhimurium* 13311 ATCC, enteropathogenic *E. coli* 0124DM, *B. cereus* B4AC, and *C. perfringens* 8239-H. All cultures, except *S. typhimurium*, were obtained from Dr. A. Hauschild (Health Protection Branch, Health and Welfare Canada, Ottawa).

C. Sample Collection, Packaging and Storage

Samples were sliced by the manufacturer and transported directly from the manufacturer to the laboratory where they

were stored at 4°C and packaged within 4 hours of collection.

Eleven slices (per pouch) of bologna were placed in aluminum-nylon-polypropylene pouches (Cryovac Division, Grace Chemicals Limited, Mississauga, Ontario). The pouches were sealed under 26 lb per sq. in. of vacuum, using either a CDL (Toronto) Model 100 vacuum packager or a KSI (Knud Simonsen Industries Ltd., Rexdale, Ontario Model 9010500) vacuum packager. Packaged bologna was stored at 4°C until analysis.

D. Sandwich Preparation, Inoculation and Storage

One teaspoon of soft margarine (Parkay) was spread on each slice of white enriched bread (McGavins Home Style). The bread with margarine (1 tsp per slice) was incubated for 18-24 hours at 21°C before the bologna was added. The bologna slices were inoculated with 0.05 ml of diluted culture using a Minitek Pipetter (BBL). The inoculum was spread over one side of the slice using a sterile glass "hockey-stick" spreader. The inoculating procedure was designed to transfer approximately 10^2-10^3 organisms per gram of bologna. One slice of inoculated bologna was placed in each sandwich. Uninoculated sandwiches were also prepared as controls.

Following inoculation, the sandwiches were placed in

"Zip Lock Seal" plastic bags (Dow Chemical) and into appropriate incubators. Three sandwiches were placed in each incubator. This procedure was followed separately for each of the 5 test organisms.

E. Bacteriological Analysis

(i) Uninoculated Controls

An 11 g wedge of bologna was cut, weighed, and analyzed as outlined in the survey section. LBS agar was omitted from the bacteriological analyses. All media were supplied by Difco, unless otherwise specified. Media used in addition to those in the survey were; Violet Red Bile agar (VRBA), with a 3-4 ml agar overlay, for E. coli; and Mannitol-Egg Yolk-Phenol Red (MYP) agar with 10 ug polymyxin per ml, prepared according to Mossel et al. (96) for B. cereus. Direct plating onto BGA was used, in addition to the salmonella procedure described previously. The VRBA and BGA plates were incubated at 36°C for 24 hours. The MYP plates were incubated at 30°C for 20-24 and 40 hours.

The uninoculated, control sandwiches were analyzed after incubation at 30°C for 25 hours. The bologna slices were weighed aseptically, by difference, and removed from the sandwich. The entire slice was homogenized in 99 ml of sterile, 0.1% peptone water. Appropriate dilutions of homogenate were plated in duplicate onto: MSA and Baird

Parker medium for staphylococci; BGA for salmonellae; VRBA for *E. coli*; MYP agar for *B. cereus*; and Egg Yolk-free TSC for *C. perfringens*.

(ii) Inoculated Sandwiches

At 0 hours (±15 minutes) an inoculated slice of bologna was homogenized with 99 ml of sterile, 0.1% peptone water and analyzed on the appropriate media. At 4, 8 and 25 hours sandwiches were removed from the incubators, and the inoculated bologna was weighed, homogenized and analyzed on the appropriate media. This procedure was repeated for each of the 5 test organisms.

(iii) Confirmation Tests

S. aureus was counted and confirmed as described in the survey methods. *S. typhimurium* was confirmed by picking BGA positive colonies to MacConkey agar and triple-sugar-iron (TSI) agar slants. MacConkey and TSI positive cultures were tested serologically with Salmonella O Antiserum Poly A-I and Salmonella O Antiserum Group B Factors 1, 4, 5 and 12 ("Serological Identification of Salmonella." Difco Laboratories, Detroit, Michigan). Enteropathogenic *E. coli* was confirmed by picking suspect colonies to MacConkey agar. MacConkey positive cultures were picked to EC medium and incubated at 45.5 ± 0.05°C for 24 and 48 hours. *B. cereus* was confirmed by catalase production and Gram stain, noting

morphology and spore type (27). C. perfringens was confirmed by the absence of catalase and Gram stain. In addition, suspected colonies were picked to nitrate-motility medium supplemented with 0.5% glycerol and 0.5% galactose (56).

F. Preparation and Maintenance of Test Cultures

All cultures, except C. perfringens, were maintained at 4°C on tryptic soy agar (TSA). C. perfringens was maintained frozen, in cooked meat medium. Cultures on TSA, except B. cereus, were subcultured bi-weekly onto fresh TSA. B. cereus was subcultured weekly to maintain optimum viability.

Aerobic cultures were prepared for inoculation by growing in tryptic soy broth (TSB) at 35°C for 20-24 hours in a shaking incubator, TSB cultures were subcultured to fresh TSB at least once before use. Inocula were prepared by diluting cultures to obtain $3x10^3$ to $3x10^4$ organisms per ml in sterile, 0.1% peptone water.

C. perfringens was grown in cooked meat (CM) medium at 36°C for 24 hours. Cultures were subcultured to fresh CM medium at least once before use. The inocula were prepared as described for aerobic cultures.

Test cultures were run as positive controls on all selective, differential and confirmation media each week.

G. Temperature Equilibration of Sandwiches in Incubators

The temperature equilibration rate of bologna slices in a packaged sandwich was measured for each incubator using a Speedomax W Multipoint Recorder and Copper-Constantan thermocouples. Two thermocouples were hooked onto each slice of bologna so that one thermocouple was located approximately 1/2 inch from the center of the slice and the other thermocouple was located approximately 3/4 inch from the edge of the slice.

H. Determination of Physical and Chemical Properties

Bologna pH was measured using the combination electrode technique described in the bologna survey section. The percent moisture was determined by drying duplicate bologna samples in a forced air oven at 110°C for 18 hours. Nitrite content (rate of addition), NaCl content, and fat content data was supplied by the manufacturers.

3. Justification of Methods

The MPN method for coliform and fecal coliform determinations, the Baird-Parker medium method for coagulase-positive staphylococci determination, and the nutrient broth, selenite-cystine, brilliant green-bismuth sulfite procedure for Salmonella are accepted methods of the Health Protection Branch, Health and Welfare, Canada

(59,60,61). These are methods used by this agency for regulation of the food industry, and therefore they have direct application and relevance to industry and this study.

The MSA procedure for enumeration of coagulase-positive staphylococci was also used because of its wide acceptance (7,22,36,44,74,87,90,114,118). However, Collins-Thompson *et al.* (24) reported that Baird-parker medium was the best available medium for enumeration of sublethally heated food poisoning strains of *S. aureus*. The Health Protection Branch (59) recommends the use of Baird-Parker medium for the enumeration of coagulase-positive staphylococci in food.

VRBA was used in the inoculation study to differentiate and enumerate enteropathogenic *E. coli*. EC broth used to confirm VRBA colonies was incubated at 45.5°C in the inoculation study. In the survey, EC broth was incubated at 44.5° ± 0.05°C. The 44.5°C temperature was used to increase recovery possibilities (i.e. to eliminate false negative results). Mehlman *et al.* (88) reported that EC broth incubated at 41.5°-44.5°C recovered as few as 100 cells/ml. To rule out false negatives, confirmation tests were performed on EC positives from the survey. The 45.5°C incubation temperature was used in the inoculation study to reduce the possibility of false positive results. Hall *et al.* (52), in a study of coliforms in market foods, reported that only 40.4% of EC positive tubes incubated at 44.5°C

contained E. coli. Thatcher and Clark (134) reported that there were three times more false positives at 44.5° than at 45.5°C. The enteropathogenic E. coli culture used was gas positive in EC broth at 45.5°C.

B. cereus was enumerated on mannitol, egg-yolk, phenol-red, polymyxin (MYP) agar developed by Mossel et al. (96). MYP has been used successfully by others (96,123,134). An alternative medium, egg-yolk polymyxin medium, developed by Kim and Goepfert (80) was described only as comparable to MYP for recovery of low levels of B. cereus from foods (80). Plates were counted after incubation at 30°C for 20-24 and 40 hours. Double counting reduced the problems of excessive spreading by B. cereus colonies, and aided in counting plates which were heavily contaminated with mannitol positive organisms (e.g. low dilution plates).

Several media have been proposed for the selection and enumeration of C. perfringens (6, and cited by 53,55,56,57,83). Hauschild and Hilsheimer (56) after evaluating SFP (Shahidi-Ferguson perfringens), TSC, Egg-yolk (EY) free TSC, and OPSP (oleandomycin-polymyxin-sulfadiazine perfringens) agars, stated that only TSC and EY-free TSC were selective enough to ensure confirmation without interference from facultative anaerobes.

Lactic acid bacteria were enumerated on LBS (18,19,25,41,77,93,110,118) and NAP (32) media. Rogosa et

al. (109), the developers of LBS agar, described it as selective for oral, vaginal and fecal lactobacilli. Davidson and Cronin (32) stated that LBS medium was unsatisfactory for bologna and certain other foods which contain large populations of streptococci or other lactics. Mol et al. (93) stated that many strains of streptobacteria, which appeared to be of major importance in vacuum packaged cooked meats, failed to grow on LBS agar. NAP agar was developed by Davidson and Cronin (32) to recover the wider range of lactic acid bacteria.

STAA medium (40) was used for the enumeration of M. thermosphactum, on the recommendation of Gardner and Kitchell (41). Gardner and Kitchell (41) also recommended analysis for enterococci when examining cured meats. A variety of media have been used to determine enterococci in foods (cited in ref. 99). Hall et al. (51) stated that KF Streptococcus medium was satisfactory. Oblinger (99) recommended Azide Blood agar, and stated that if KF Streptococcus medium was used that representative colonies should be confirmed. In Oblinger's study (99) recoveries of enterococci in bologna with KF Streptococcus agar were ca. 66% of recoveries with Azide Blood agar. Thatcher and Clark (134) recommended a 3-stage procedure: presumptive enumeration on Crystal-Violet Azide Blood agar; confirmation on Thallous Acetate Tetrazolium Glucose agar; and identification of species. KF Streptococcus agar was chosen

for use in this study for convenience.

A minimum volume of inoculum was desired in the inoculation study. Preliminary work determined that 0.05 ml was the minimum volume which could be spread over an entire side of a slice of bologna. When 0.025 ml was used, the inoculum was absorbed into the slice before it could be spread over the entire surface.

Samples were homogenized in, and serial dilutions were made using sterile, 0.1% peptone water. Oblinger and Kennedy (100) reported no statistical difference for bologna counts using five different diluents: Butterfield's diluent, 0.5% peptone, 0.1% peptone, saline and distilled water.

By analyzing just the bologna slice rather than the entire sandwich, in the inoculation study, an under estimation of pathogen numbers could occur if organisms were absorbed onto the bread. It was felt, however, that absorption onto the bread would be negligible because the inoculum was absorbed into the bologna before the top slice of bread was put in place, and because the margarine would provide a partial barrier to absorption by the bread. In any event, an error of this type would be constant throughout the experiment and should not affect the interpretation of the results.

Chapter IV. Results and Discussion

1. Bologna Survey

Table 1 indicates the source of bologna samples analyzed in this study. Because of limitations at retail stores, not all cells in the survey design could be evenly represented. The apparent over-sampling of some manufacturers, and under-sampling of others was in fact a measure of the distribution of bologna brands in the marketplace. Only two of the five small stores (i.e. open chest display) carried brands other than Gainers'. One of these handled Gainers' and Burns' bologna, while the other sold only Canada Packers' product.

The distribution of bologna pH values is shown in Table 2. The values ranged from 4.9 to 6.8. Almost 50% of the samples were in the pH range 6.1 to 6.5, and 22% had a pH of 5.5 or lower. Bologna pH has been reported previously as 6.2 to 6.4 (122), 5.2 to 5.3 (105) and 5.0 to 6.5 (77). The wider range reported by Kempton and Bobier (77) was attributed to age effects. They noted that pH began to drop after 4 weeks of refrigerated storage. Age was shown to have a significant effect ($p \leq 0.0019$) on pH in this study. New bologna generally fell in the pH range 6.1 to 6.5, while old bologna often had pH values below 5.5. However, approximately 55% of old samples failed to develop low pH. This might have been due to differences in manufacturer or

Table 1. Source of samples obtained for analysis in survey of bacteriological quality of vacuum packaged, sliced bologna.

Display type	Manufacturer*						
	A	B	C	D	E	F	Total
	Number "new" (≤14 days from manufacture) samples						
Peg board	4	6	2	5	5	-	22
Open chest	2	-	3	-	-	-	5
Open case of peg board	-	-	1	-	-	5	6
	Number "old" (≥22 days from manufacture) samples						
Peg board	1	2	1	2	6	-	12
Open chest	7	-	17	-	6	-	30
Open case of peg board	1	-	3	-	-	3	7
Laboratory	5	5	5	6	5	5	31
Total	20	13	32	13	22	13	

* A - Burns Meats Limited (Establishment 1A)
  B - Inter-Continental Packers (Establishment 69)
  C - Gainers Limited (Establishment 27)
  D - Swift Canadian Co. Ltd. (Establishment 18B)
  E - Canada Packers Ltd. (Establishment 7A)
  F - J.M. Schneider Inc. (Establishment 35)

Table 2. pH profiles of bologna samples included in the survey.

Bologna type	pH					
	4.6-5.0	5.1-5.5	5.6-6.0	6.1-6.5	6.6-7.0	Total
	number (percent) of samples					
All samples	3 (2.7)	21 (18.9)	18 (16.2)	54 (48.6)	15 (13.5)	113
"New" samples	0	2	6	27	1	36
"Old" samples	3	19	12	27	14	65
Manufacturer*						
A	0	2	5	11	0	18
B	0	0	3	9	1	13
C	3	17	3	8	1	32
D	0	2	2	9	0	13
E	0	0	2	7	13	22
F	0	0	3	10	0	13

* A - Burns Meats Limited (Establishment 1A)
  B - Inter-Continental Packers (Establishment 69)
  C - Gainers Limited (Establishment 27)
  D - Swift Canadian Co. Ltd. (Establishment 18B)
  E - Canada Packers Ltd. (Establishment 7A)
  F - J.M. Schneider Inc. (Establishment 35)

manufacturer-age interaction. New and old Canada Packers' bologna generally had a high pH, while old Gainers' bologna usually had a low pH. These manufacturer and age effects might explain the narrow pH ranges reported by Riemann *et al.* (105) and Steinke and Foster (122).

Product pH has a significant effect on pathogen development (10,23,42,64,71). *S. aureus* was inhibited in anaerobically packaged fermented sausage at pH 5.7 (10), and in cured meat stored at 10°C at pH 5.6 (42). *C. perfringens* was inhibited at pH 6.1 (64), and *S. typhimurium* was inhibited in fermenting sausage stored at 30°C at pH 5.1 (23). The variabilty in bologna pH, therefore, represents an important observation in this study,

The distribution of saprophyte (total bacteria) counts in bologna was also highly variable (Table 3). This variability is significant in that pathogens would be exposed to different levels of competition. Total counts at 21°C and 36°C ranged up to 1.1×10^9 and 1.0×10^9 organisms per gram, respectively. Approximately 50% of the samples at 21°C and 40% at 36°C had counts above 1.0×10^7 organisms per gram. Total counts at 4°C ranged up to 9.4×10^8 organisms per gram, with 36% of the counts above 1.0×10^7 organisms per gram. Maximum total counts reported in the literature are 10^7 - 10^8 organisms per gram (2,63,70,77,139). The two samples in the study with counts of 10^9 organisms per gram

Table 3. Profiles of saprophytic and indicator organisms for the bologna samples.

Bacteria counts[1]	Range of Bacteria Counts per gram											
	0^*	10^0	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9	Total Samples
	Number (percent) of samples											
LST	49 (43.3)	58 (51.4)	6 (5.3)	0	0	0	0	0	0	0	0	113
BGB	108 (95.5)	4 (3.6)	1 (0.9)	0	0	0	0	0	0	0	0	113
NAP	10 (22.7)	-	0	4 (9.1)	2 (4.5)	4 (9.1)	2 (4.5)	3 (6.8)	11 (25.0)	8 (18.2)	0	44
LBS	33 (29.7)	-	13 (11.7)	12 (10.8)	12 (10.8)	5 (4.5)	4 (3.6)	15 (13.5)	15 (13.5)	2 (1.8)	0	111
SPC 36°C	0	-	2 (1.8)	14 (12.4)	14 (12.4)	10 (8.8)	16 (14.2)	10 (8.8)	24 (21.2)	22 (19.5)	1 (0.9)	113
SPC 21°C	0	-	0	9 (7.9)	15 (13.3)	7 (6.1)	15 (13.3)	9 (7.9)	26 (23.1)	31 (27.6)	1 (0.9)	113
SPC 4°C	0	1 (0.9)	12 (10.6)	10 (8.8)	11 (9.7)	13 (11.5)	13 (11.5)	12 (10.6)	30 (26.7)	11 (9.7)	0	113
KF	25 (22.1)	-	19 (16.8)	6 (5.3)	9 (8.0)	8 (7.1)	10 (8.8)	10 (8.8)	20 (17.7)	6 (5.3)	0	113
STAA	71 (62.8)	-	-	8 (7.1)	11 (9.7)	8 (7.1)	7 (6.2)	7 (6.2)	1 (0.9)	1 (0.9)	0	113

[1] Interpretation of media codes indicated in text pp. 41-42
* No detectable organisms at lowest dilution

might not be statistically different from published maxima, or might indicate poor vacuum packaging. Ingram (70) and Roth and Clark (110) reported that the maximum population in vacuum packaged meats rarely exceeded 10^8 organisms per gram, while that of aerobically packaged product often exceeds 10^9 organisms per gram. In any event, this study indicated that self-limiting maximum bacterial populations, above 10^7 organisms per gram were common and might be expected in vacuum packaged bologna. The minimum total counts observed were slightly lower than those previously reported (126,139).

Age had a significant effect ($p \leq 0.001$) on total counts at all incubation temperatures, with new bologna having significantly lower counts. Warnecke *et al.* (139) and Hill *et al.* (63) reported a similar relationship between total counts and age. The effect of manufacturer on total counts was significant at 36°C ($p \leq 0.0004$) and at 21°C ($p \leq 0.0028$), but not at 4°C ($p \geq 0.074$). Gainers' bologna frequently had high total counts at 36°C, while Canada Packers' product often had low counts at 21°C.

Comparison of total counts at the different incubation temperatures suggests the storage history of the samples (126). Most psychrophiles grow at 4° and 21°, but not at 36°C (121), while mesophiles develop at 21° and 36°, but not at 4°C (4,121,134). Therefore, samples which have been

stored continuously at 4°C or lower should have total counts at 4° and 21°C which are higher than total counts at 36°C. Table 4 indicates that 40% of the samples had total counts which were at least 5 times higher at 4° and 21° than at 36°C. In comparison, samples which have been exposed to prolonged storage above 10°C should have totals counts at 21° and 36°C which are higher than totals counts at 4°C. Total counts at 21° and 36°C were at least 5 times higher than at 4°C for 44% and 29% of the samples, respectively (Table 4). Thus, 40% of the samples appeared to have been stored with adequate refrigeration, while 29% to 44% of the samples appeared to have been exposed to unsatisfactory storage, such as prolonged holding at 10°C or above.

In addition to total counts, levels of specific saprophytes, which have been reported to predominate the microflora of vacuum packaged, cooked, cured meats (2,3,62,77,93,97,102,119,122,139) were also measured in this study (Table 3). Lactic acid bacteria (NAP count) were detected at levels up to 6.1×10^8 organisms per gram, with 45% of the samples having counts above 10^7 organisms per gram. Lactic acid bacteria were not detected in 22.7% of the samples. Lactic acid bacteria have been reported as the major constituent of vacuum packaged meat microflora (2,3,11,77,122). Lactobacilli (LBS count) were detected at lower levels than total lactic count, with only 15.3% of the samples having counts above 10^7 organisms per gram, and with

Table 4. Comparison of Standard Plate Counts at 4°, 21° and 36°C for bologna samples analyzed.

	Percent of samples at least 5 times greater at higher incubation temperature	
	4°C	21°C
21°C	44	-
36°C	29	2.7
	Percent of samples at least 5 times lower at higher incubation temperature	
	4°C	21°C
21°C	2.7	-
36°C	40	40

a maximum count of $1.1x10^8$ organisms per gram. Lactobacilli were not detected in 29.7% of the samples. These results supported the findings of Davidson and Cronin (32) and Mol *et al.* (93) that LBS agar was not satisfactory for the enumeration of lactic acid bacteria in bologna. However, the correlation (Table 5) between NAP and LBS (r=0.786) indicated that lactobacilli were a consistent constituent of the lactic acid bacteria population. The strong correlation between NAP count and total counts at 21°C (r=0.823) and at 36°C (r=0.814) suggested that these total counts might be a measure of lactic acid bacteria in bologna. The poorer, though still significant, correlation (r=0.473) between NAP count and total count at 4°C was surprising in that Allen and Foster (2), Bartl (11) and Kempton and Bobier (77) reported that lactic acid bacteria predominated the microflora of vacuum packaged cured meats after refrigerated storage. The LBS count was more highly correlated (r=0.644) with total count at 4°C, indicating, as reported by Alm *et al.* (3), more predictable lactobacillus growth after refrigerated storage. As expected, bologna pH showed a strong (negative) correlation with lactic acid bacteria (r=-0.664). The influence of age and manufacturer on pH might be responsible for this correlation not being higher. Kempton and Bobier (77) reported that pH did not begin to drop significantly until after maximum lactic acid bacteria populations were reached, and that at least

Table 5. Pearson's correlation coefficients (98) between pH and various saprophytic bacterial counts[1] on bologna.

Variables	pH	SPC 36°C	SPC 21°C	SPC 4°C	NAP	LBS	KF
SPC 36°C	-0.604						
SPC 21°C	-0.550	0.894					
SPC 4°C	-0.357	0.606	0.730				
NAP	-0.664	0.814	0.823	0.473			
LBS	-0.618	0.662	0.649	0.644	0.786		
KF	-0.597	0.688	0.625	0.336	0.571	0.469	
STAA	0.164	-0.125	-0.040	0.034	-0.421	-0.154	-0.154

[1] SPC - Standard Plate Count
NAP - Lactic acid bacteria
LBS - Rogosa *et al.* Lactobacillus medium
KF - Group D Streptococcus count
STAA- *M. thermosphactum* medium

2 weeks refrigerated storage were required before lactic acid bacteria predominated. This study also indicated that age of product affected lactic acid bacteria (NAP) counts, including lactobacilli (LBS), counts ($p \leq 0.0005$)[1] , with new bologna having lower counts. Manufacturer effects on lactic acid bacteria were also significant ($p \leq 0.078$). Canada Packers' bologna generally had lower counts, while Gainers' product generally had higher lactic acid bacteria counts. Differences in the microflora of equipment were probably responsible for these manufacturer effects (77,93).

M. thermosphactum, contrary to expectations (4,31,41,110,127), was detected in only 37% of the samples, with only 1.8% of samples having counts above 10^7 organisms per gram. Correlations between M. thermosphactum and all other parameters measured were very low (Table 5), suggesting that this organism was of minor significance in vacuum packaged bologna in this study.

Group D streptococci and coliform organisms are commonly used as indicators of fecal contamination (17,134). In this study, presumptive (LST) and confirmed (BGB) coliforms were present in only 56.7% and 4.5% of the samples, respectively (Table 3). Fecal coliforms were not detected in any samples. The maximum confirmed coliform

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[1] Effects of age on NAP counts were not significant by the chi-square analysis ($p=0.3$).

count was 43 organisms per gram. The lowest concentration detectable by the MPN procedure was 3 organisms per 10 grams. In comparison, Group D streptococci (KF) were detected in 78% of the samples (Table 3), with a maximum count of 2.1×10^8 organisms per gram. Only 40% of the samples had counts below 100 organisms per gram. Poor correlation between Group D streptococci and coliforms has been reported previously (17,134).

S. durans was confirmed in 48.7% of the Group D streptococci isolates (Table 6). S. faecalis and S. faecium, which are considered indicators of fecal contamination (17,134), represented 5.0% and 37.8% of isolates. Buttiaux and Mossel (17) reported that direct fecal contamination resulted in less than 10 Group D streptococci per gram of food. Storage temperature abuse permitting growth, or contamination from equipment, therefore, is suggested where counts are above 10^2 Group D streptococci per gram. The absence of fecal E. coli suggested that direct fecal contamination might not be involved. The frequent occurrence of S. durans, amoung Group D streptococci, was supported by the literature, which suggested that S. durans was associated with heat processed meats (124), and supported the suggestion of contamination from equipment.

The Group D streptococcus count showed poor correlation with, and was usually lower than, the lactic acid bacteria

Table 6. Speciation of Group D Streptococcus isolates (125).

Organism:	*S. faecalis*	*S. faecium*	*S. durans*	Not identified
Number (percent)	6 (5.0)	45 (37.8)	58 (48.7)	10 (8.4)

count. Correlation with pH was significant (r=-0.597) but less than the 0.7 value necessary to achieve 50% predictability. Group D streptococci were correlated significantly with total count at 36°C (r=0.688). This correlation supported the suspicion of temperature abuse. Manufacturer had a significant effect on Group D streptococci ($p \leq 0.0001$), with Gainers' bologna often having a higher count and Inter-Contenental Packers' product having a lower count. The significance of age effects on Group D streptococci was not clear (Table 8a).

Levels of potential pathogens were low in all samples (Table 7). Neither salmonellae nor E. coli were detected. Only 10 samples (9.0%) were positive for clostridia, at levels up to 1.6×10^2 organisms per gram. Only 1 sample was positive for S. aureus , at 1.0×10^2 organisms per gram, on Baird-Parker medium. On MSA, 56 samples (56%) were positive for presumptive staphylococci, at levels up to 1.4×10^5 organisms per gram. The differences between MSA and Baird-Parker medium counts are significant, and may result from MSA supporting the growth of non-S. aureus but salt tolerant, mannitol positive organisms (7,73).

This low incidence of pathogen isolation compared well with the published information, which indicated that 93-100% of bologna and frankfurters were negative for S. aureus (62,74,126); 100% were negative for salmonellae

Table 7. Profiles of potentially pathogenic organisms in bologna samples.

Organism	Bacteria counts (range)/gram						Total Samples
	0*	10^0	10^1	10^2	10^3	10^4	10^5	
	Number (percent) of samples							
Clostridia	93 (91.0)	6 (5.4)	2 (1.8)	2 (1.8)	0	0	0	113
Salmonellae	113 (100)	0	0	0	0	0	0	113
S. aureus (BP)	112 (99.1)	-	0	1 (0.9)	0	0	0	113
Staphylococci (MSA)	44 (44.0)	-	17 (17.0)	29 (29.0)	8 (8.0)	1 (1.0)	1 (1.0)	100
E. coli type I	113 (100)	0	0	0	0	0	0	113

* No detectable organisms at lowest dilution

(79,126,140); and only 0.1% of frankfurters were positive for *E. coli* (126). This might be due to a low level of contamination or to inhibition of pathogen growth by one or more of the inhibitors found in vacuum packaged cured meats (e.g. nitrite, salt, pH, oxygen partial pressure, competition and temperature).

In addition to the survey of microbial content and pH of bologna, this study also allowed the influence of manufacturer, age and display type to be analyzed. Because of difficulties in obtaining a homogeneous design (Table 1), a factorial analysis of variance could not be performed. Instead, the chi-square analysis for independence (98) was used to determine the significance of manufacturer, age and display, independently (Table 8a). Also, using results from new bologna samples (all brands) and old bologna samples (all brands after "laboratory storage" to expiry date), it was possible to perform an analysis of variance (98) which considered the effects of manufacturer, age, and their interaction. The results of these analyses are presented in Tables 8a and 8b. There was generally good agreement between the chi-square and the analysis of variance results, except for age effects on NAP and Group D streptococcus counts. The reasons for this were not indicated by the data, however they might be due to interaction effects of display or to the different sample populations which are considered in the two analyses.

Table 8a. Significance (Probability) of manufacturer, age of product and display type effects on bacterial counts and pH of bologna samples.

Bacterial count[1] and pH	Manufacturer Chi square[2]	Manufacturer ANOVA[3] F-test	Age Chi square[2]	Age ANOVA[3] F-test	Display Chi square[2]
NAP	0.0078	0.0002	0.3324	0.0005	0.0229
SPC 36°C	0.0000	0.0004	0.0000	0.0014	0.0001
SPC 21°C	0.0013	0.0028	0.0000	0.0005	0.0005
SPC 4°C	0.0813	0.0740	0.0000	0.0001	0.0044
KF	0.0000	0.0001	0.1042	0.0004	0.0001
LBS	0.0004	0.0001	0.0002	0.0001	0.2779
STAA	0.0457	0.0440	0.2055	0.6200	0.5819
pH	0.0000	0.0001	0.0019	0.0003	0.0113

[1] SPC - Standard Plate Count
NAP - Lactic acid bacteria
LBS - Rogosa et al. Lactobacillus medium
KF - Group D Streptococcus count
STAA M. thermosphactum medium
[2] Probability of chi-square analysis being significant
[3] Probability of F-test by ANOVA (98) analysis being significant. Only "new" and "laboratory" stored samples were included in this analysis.

Table 8b. Significance of manufacturer x age interaction effects on bacterial counts and pH of bologna samples.

Bacterial count[1] or pH	Probability of F-test by ANOVA[2]
NAP	0.200
SPC 36°C	0.389
SPC 21°C	0.433
SPC 4°C	0.973
KF	0.114
LBS	0.137
STAA	0.072
pH	0.002

[1] See Table 8a, p. 70 for media codes
[2] The ANOVA analysis (98) included only "new" and "laboratory" stored samples

To determine which of the variables (i.e. manufacturer, age and display type) were contributing to independence, chi- square values for the individual cells within each overall chi-square were determined (142). Tables 9a-c list the individual cells which contributed a major portion of the overall chi-square value, and which therefore, contributed significantly to independence of the variable. In addition to this chi-square breakdown, the Duncan's Multiple Range Test (98), using only the results from samples stored in the laboratory to expiry date, ranked manufacturer by specific bacterial count and by pH (Table 10).

Manufacturer had a significant effect ($p \leq 0.01$) on total counts at 21° and 36°C, Group D streptococcus count, LBS count, NAP count and pH. The significant difference was due to Canada Packers', Inter-Continental Packers' and Gainers' having significantly more than the theoretically expected number of samples falling into specific cells in the Chi Square analyses (Table 9a). Canada Packers' samples frequently had a low total count at 36°C, low NAP and LBS counts, and a high pH; Inter-Continental Packers' bologna frequently had a low Group D streptococcus count and an intermediate (i.e. 10^5 organisms per gram) total count at 21°C; Gainers' product frequently had a high total count at 36°C and a low pH. Other manufacturers did not differ significantly according to the chi-square analysis. The

Table 9a. Individual chi-square cells contributing to significant chi-square analyses for manufacturer.

Bacterial count[1] or pH	Individual chi-square cell[2]	Percent of overall chi-square	Degrees of Freedom	Direction of deviation from theoretical
NAP	E x 10^0-10^1/g	28	35	+
SPC 36°C	B x 10^3/g	8	35	+
	C x 10^7/g	14	35	+
	E x 10^5/g	9	35	+
SPC 21°C	E x 10^2/g	15	35	+
	E x 10^8/g	9	35	-
KF	B x 10-10^1/g	11	40	-
	C x 10^7-10^8/g	14	40	+
LBS	C x 10^6/g	18	40	+
pH	C x 4.6-5.5	27	20	+
	E x 6.6-7.0	36	20	+

[1] See Table 8a, p. 70 for media codes
[2] A - Burns Meats Limited (Establishment 1A)
B - Inter-Continental Packers (Establishment 69)
C - Gainers Limited (Establishment 27)
D - Swift Canadian Co. Ltd. (Establishment 18B)
E - Canada Packers Ltd. (Establishment 7A)
F - J.M. Schneider Inc. (Establishment 35)

Table 9b. Individual chi-square cells contributing to significant chi-square analyses for age.

Bacterial count[1] or pH	Individual chi-square cell	Percent of overall chi-square	Degrees of Freedom	Direction of deviation from theoretical
SPC 31°C	New x 10^2-10^3/g	42	8	+
	New x 10^8/g	13	8	-
SPC 21°C	New x 10^2-10^3/g	36	7	+
	New x 10^8/g	25	7	-
SPC 4°C	New x 10^3/g	21	8	+
LBS	New x 10^1, 10^3/g	34	8	+
	New x 10^7/g	14	8	-
pH	New x 6.1-6.5	33	4	+

Table 9c. Individual chi-square cells contributing to significant chi-square analyses for display type.

Bacterial count[1] or pH	Individual chi-square cell	Percent of overall chi-square	Degrees of Freedom	Direction of deviation from theoretical
	Display type[2]			
SPC 36°C	A x 10^3/g	13	24	+
	B x 10^7/g	13	24	+
SPC 21°C	A x 10^8/g	15	21	+
	D x 10^8/g	10	21	+
SPC 4°C	A x 10^3/g	9	24	+
KF	B x 10^7/g	16	24	+
	C x 10^6/g	20	24	+

[1] See Table 8a, p. 70 for media codes
[2] A - peg board retail display
B - open chest retail display
C - open case section of peg board display
D - laboratory stored samples to pull date

Table 10. Ranking of manufacturers by bacterial count and pH of their bologna product stored at 4°C in the laboratory until "pull" date.

Bacterial count[1] or pH	Duncan's multiple range test (1% confidence level) Rank order and significant differences between Manufacturers[2]					
NAP	E	B	D	F	C	A
SPC 36°C	E	B	F	D	A	C
SPC 21°C	E	F	B	D	A	C
SPC 4°C	E	F	B	D	A	C
KF	B	E	F	D	A	C
LBS	E	F	B	D	A	C
STAA	F	C	D	A	B	E
pH	C	A	D	F	B	E

[1] See Table 8a, p. 70 for media codes

[2] A - Burns Meats Limited (Establishment 1A)
B - Inter-Continental Packers (Establishment 69)
C - Gainers Limited (Establishment 27)
D - Swift Canadian Co. Ltd. (Establishment 18B)
E - Canada Packers Ltd. (Establishment 7A)
F - J.M. Schneider Inc. (Establishment 35)

Duncan's Multiple Range results (Table 10) also indicated interesting patterns of apparent manufacturer effects. All bacterial counts which were affected significantly by manufacturer (i.e. total counts at 21° and 36°C, Group D streptococcus, LBS and NAP counts) showed similar manufacturer rankings. Canada Packers', Inter-Continental Packers' and Schneiders' bologna consistently had lower bacterial counts, Swifts bologna consistently had mid-range counts, and Burns' and Gainers' products consistently had higher counts. The ranking by pH was the reverse of that by bacterial count except that Gainers' bologna had the lowest pH, while Burns' product had a mid-range pH.

Age of product had a significant effect ($p \leq 0.01$) on total counts at all incubation temperatures, LBS count and pH. New bologna had lower counts and higher pH (Table 8b). The effect of age on NAP and Group D streptococcus counts was not clear as discussed previously. The only significant manufacturer-age interaction was pH ($p=0.002$). This effect was due to the tendency for old Canada Packers' bologna to have a high pH (e.g. above 6.5) and for old Gainers' bologna to have a low pH (e.g. below 5.6).

Display type had a significant effect ($p \leq 0.01$) on total counts at all incubation temperatures and on Group D streptococcus count. However, no single display type stood out as contributing to overall high or low counts (Table

9c). Peg board samples had lower total counts at 4° and 36°C, but a higher total count at 21°C; samples from the open case section of peg boards had a lower total count at 4°C, but a higher Group D streptococcus count; samples from open chest display had a higher total count at 36°C and a higher Group D streptococcus count; laboratory stored samples had a higher total count at 21°C. The results for open case section of peg boards and open chest displays must be interpreted with caution because not all manufacturers were represented in these display types. The open case section of peg boards contained only Gainers' and Schneider's bologna, and the open chest displays contained only Gainers', Burns' and Canada Packers' product. The Gainers' product in particular, could influence the Group D streptococcus results.

The literature reviewed did not consider the effect of manufacturer or display type on the bacterial counts or the pH of bologna. Comparisons with the literature therefore, cannot be drawn. The literature considering age effects have been referred to earlier in this discussion.

2. Inoculation Study

Survey results indicated that pH, bacterial numbers (i.e. competition) and probably storage temperature were variable in bologna. However, there was no evidence of pathogen growth, even in samples with the most favorable combinations of pH and competition (i.e. high pH and low bacteria counts). This might have been due to lack of contamination during manufacture, or to the effects of other inhibitors (i.e. nitrite, salt, oxygen partial pressure). The inoculation study attempted to evaluate the effects of pH, competition and storage temperature variability on pathogen growth, under potentially dangerous, but realistic conditions. Aerobic incubation of inoculated samples eliminated inhibition due to reduced oxygen partial pressure (i.e. for aerobic pathogens).

For the inoculation study, bologna intended to represent maximum and minimum levels of pH and competitive inhibition of pathogens, was chosen on the basis of survey results. The effects of pH were evaluated using old Gainers' product (probable pH of 5.5 or below) and old Canada Packers' product (probable pH near 6.5). Old bologna was used to provide relatively constant levels of competition: Appendix II indicates that all old bologna samples had total counts above 10^6 organisms per gram. Competitive effects were determined using new and old Canada Packers' bologna.

The pH was similar in both age types, but bacterial counts were consistently lower in new bologna (Appendix II). As a result of storage depletion, the nitrite level in old bologna was probably 15-20% lower than in new product (115).

The growth of test organisms in bologna sandwiches is shown in Figures 1 to 5. The most striking observation was that significant growth occurred only after incubation at 30°C for 25 h. All test organisms, except C. perfringens, grew on high pH and both age types of bologna after long incubation. C. perfringens not only failed to grow on any sample, but appeared to decline after exposure to the more abusive storage conditions, especially in low pH bologna (Figure 5). This decline was probably due to the presence of oxygen, or a combination effect with other inhibitors (64,137). Greater decline at higher temperatures suggested that an active toxic mechanism might be involved. Dempster (34) reported that temperature affected the velocity of the bactericidal action of salt. Inhibition of spore germination by nitrite was not indicated, as the C. perfringens inoculum was an actively growing, vegetative culture.

Pathogen growth, after incubation at 30°C for 25 h, is ranked in Table 11. S. aureus and B. cereus grew sufficiently on new and old (high pH) bologna to present a health hazard. Enteropathogenic E. coli and possibly

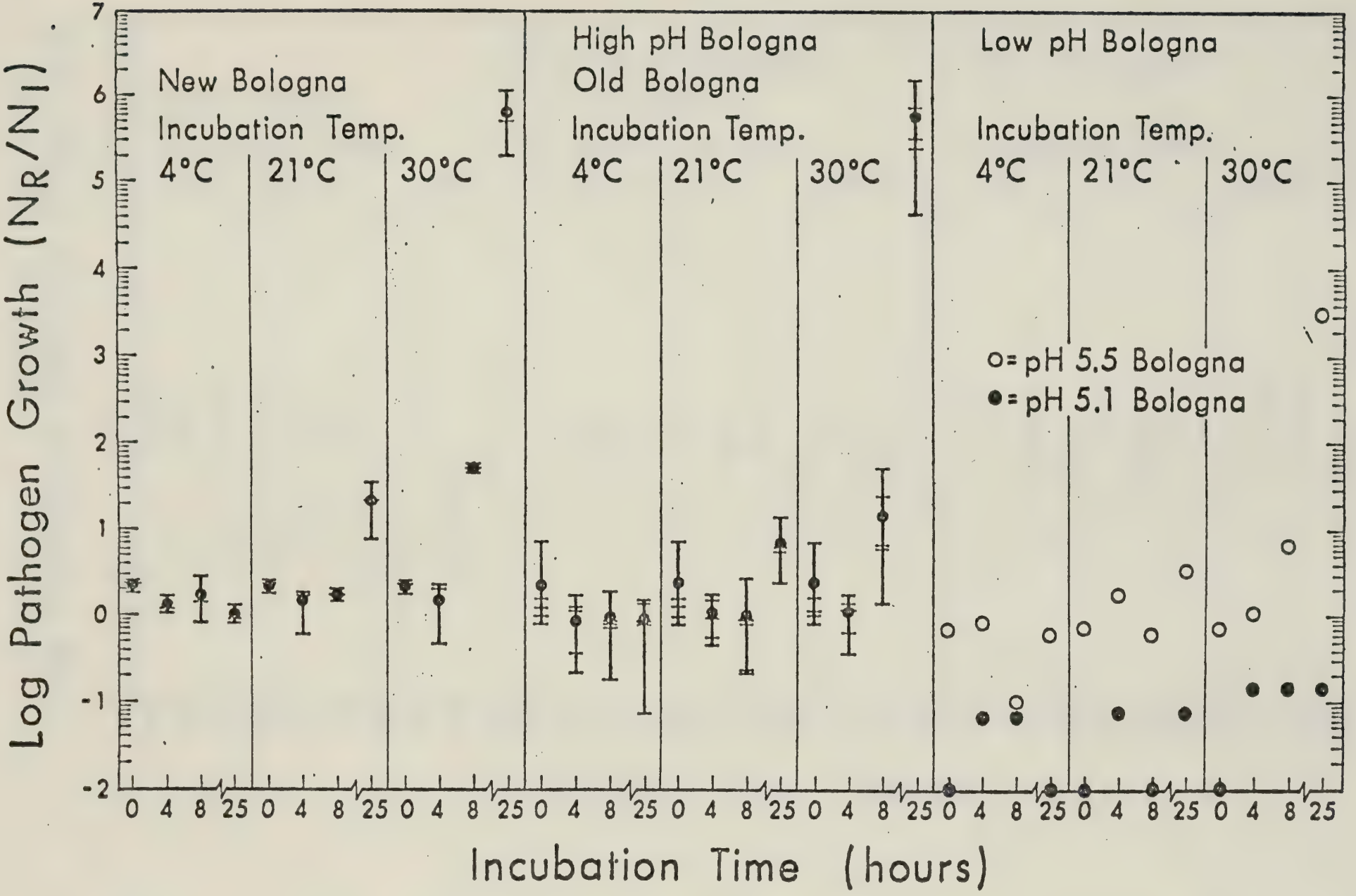

Figure 1. Influence of time and temperature of incubation, age and pH of bologna on growth of S. aureus.

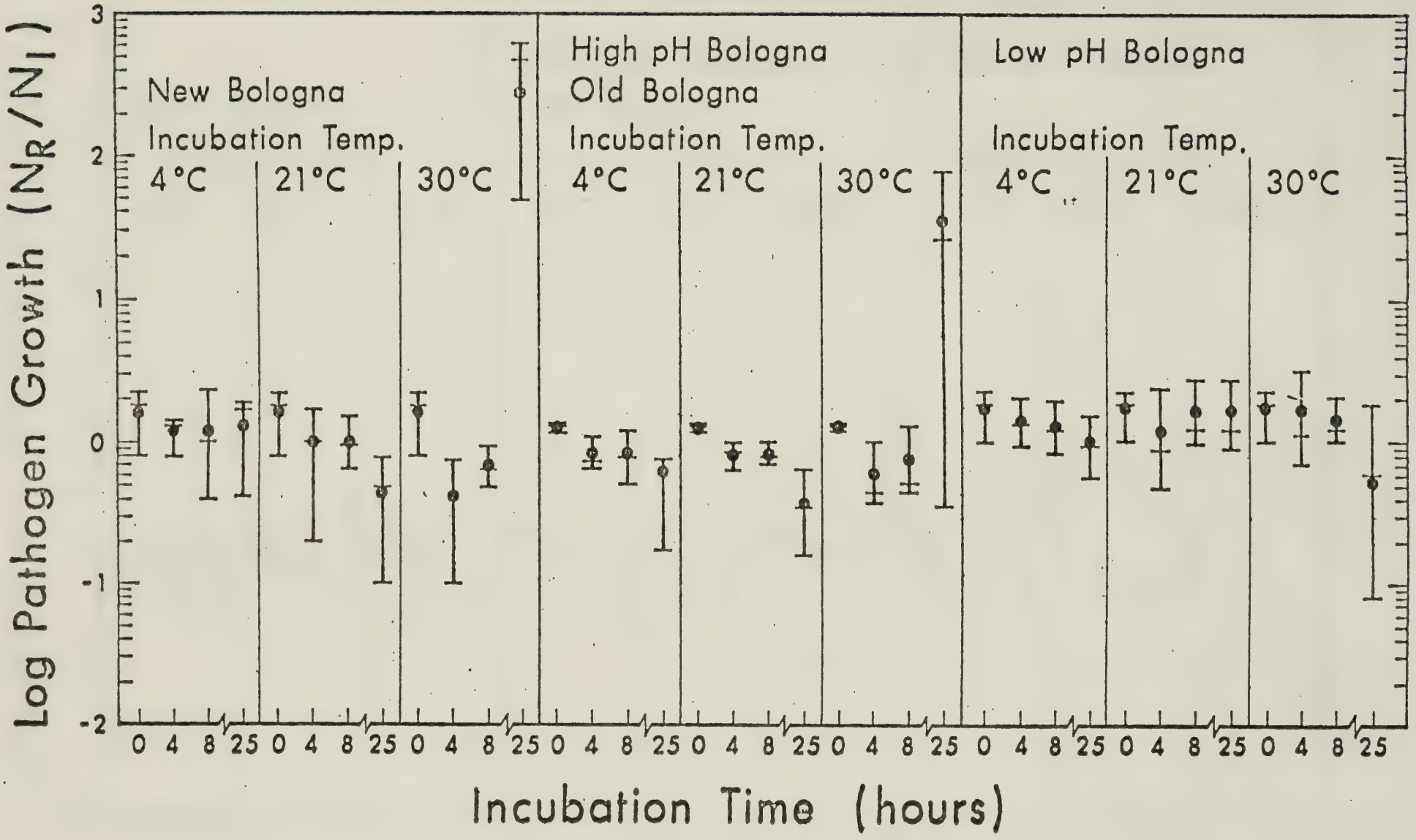

Figure 2. Influence of time and temperature of incubation, age and pH of bologna on growth of S. typhimurium.

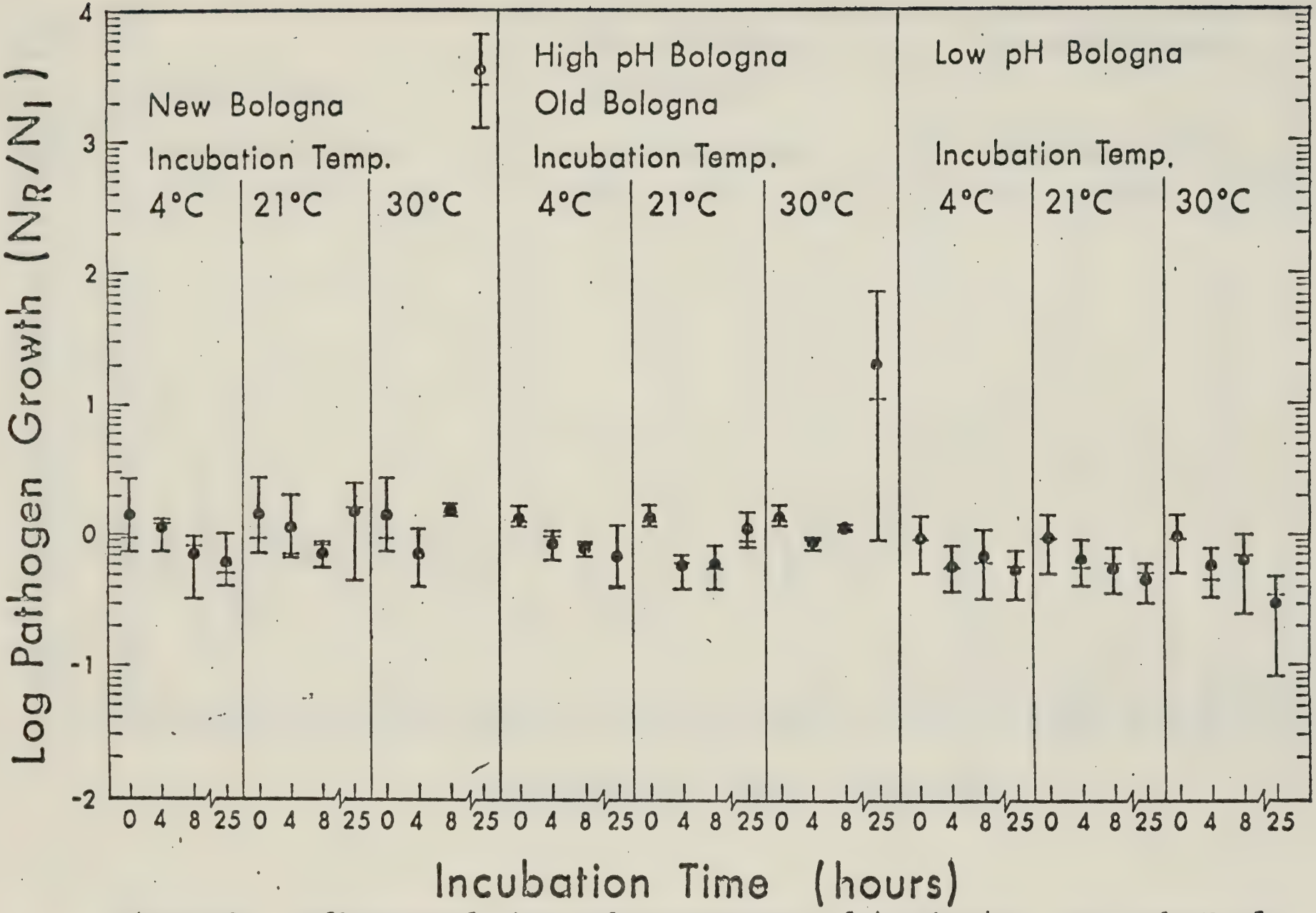

Figure 3. Influence of time and temperature of incubation, age and pH of bologna on growth of E. coli.

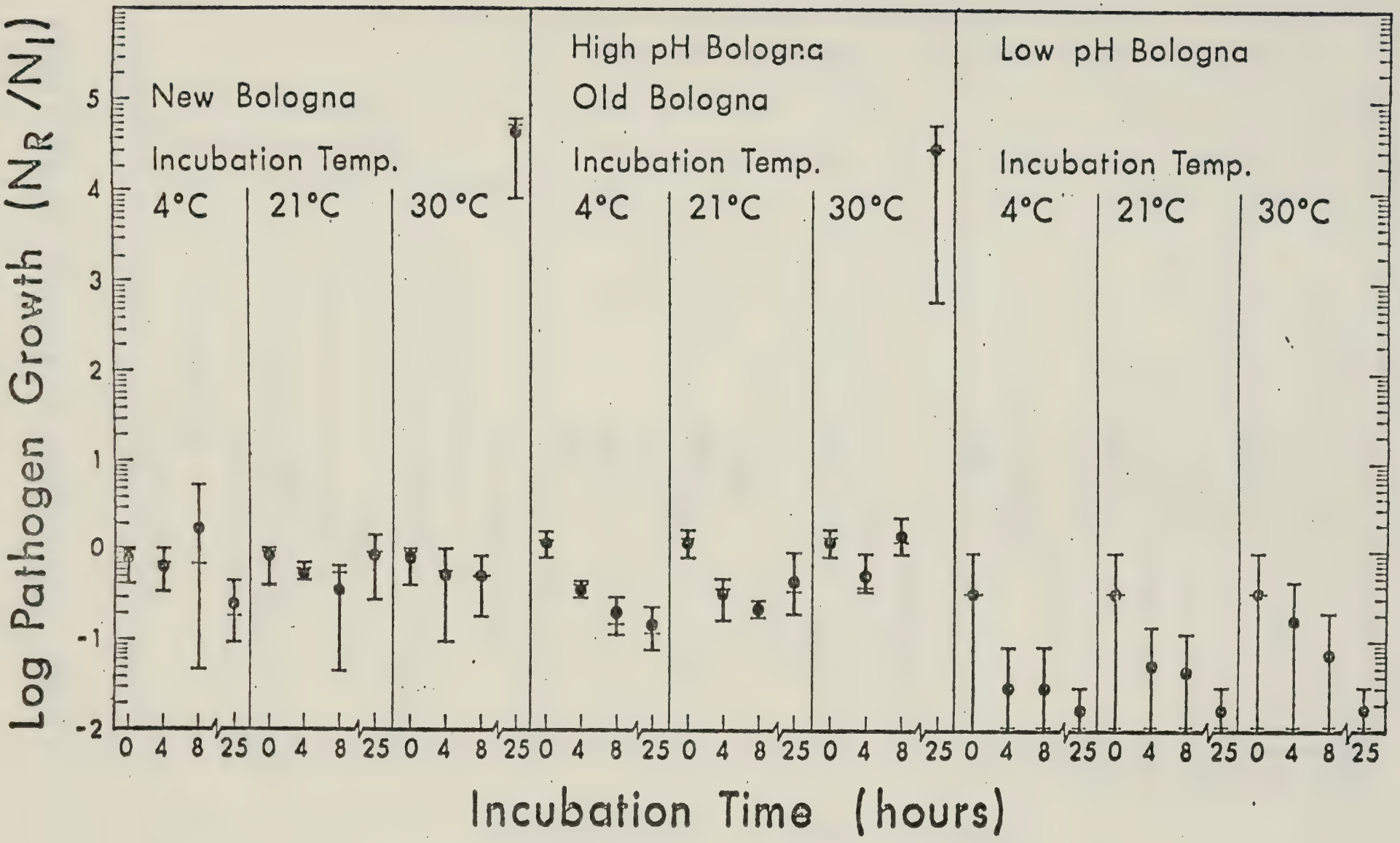

Figure 4. Influence of time and temperature of incubation, age and pH of bologna on growth of B. cereus.

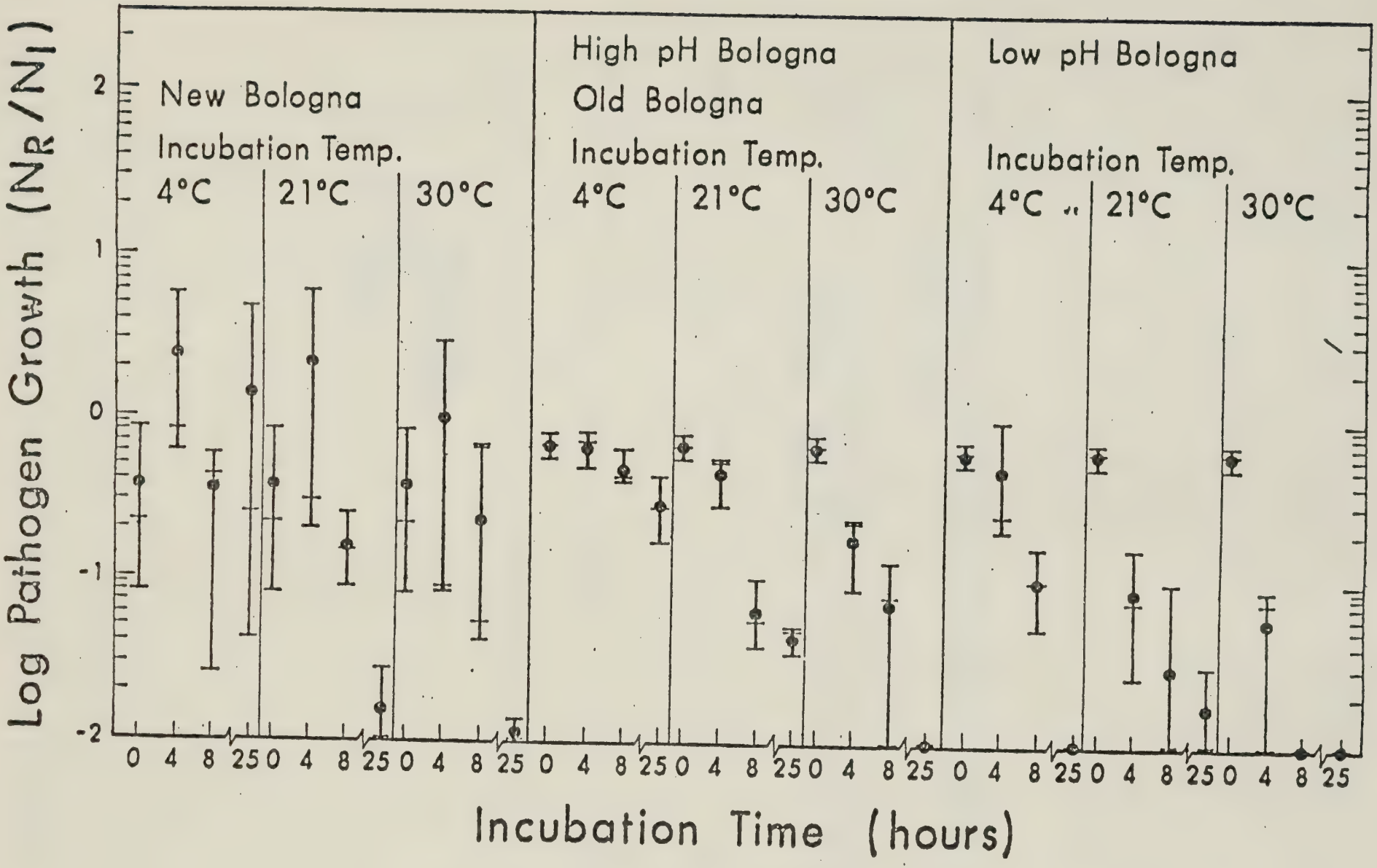

Figure 5. Influence of time and temperature of incubation, age and pH of bologna on growth of C. perfringens.

Table 11. Ranking of pathogenic test organisms[1] by ability to grow on bologna in sandwiches, stored at 30°C for 25 hours.

Bologna type	Duncan's multiple range test (5% confidence level). Rank order and significant difference between counts				
New	Cp	St	Ec	Bc	Sa
Old (High pH)	Cp	St	Ec	Bc	Sa

[1] Cp - C. perfringens
St - S. typhimurium
Ec - E. coli
Bc - B. cereus
Sa - S. aureus

S. typhimurium only increased to potentially dangerous levels on new bologna (high pH). The greater growth of S. aureus and B. cereus was probably due to their greater salt tolerance (137). The relatively poor growth of S. typhimurium and enteropathogenic E. coli was not surprising. Heiszler et al. (62) reported that curing agents were selective for Gram positive organisms.

Under conditions less abusive than 30°C for 25 h, S. aureus was the only pathogen that grew (Figure 1). Growth was less than a 100-fold population increase in all cases, and therefore, unless initial contamination was heavy (e.g. 10^4-10^5 organisms per gram), would not represent a food poisoning potential. There was no growth of any of the pathogens after 4 h at any of the incubation temperatures, after 8 h at 4°C or 21°C, or after 25 h at 4°C. The lack of growth at 4°C was expected (5,47,64,128,137). Prolonged lag phase, after exposure to levels of inhibitors found in cured meats, has been reported for S. aureus (34,76,137) and salmonellae (137). Genigeorgis et al. (42) observed enterotoxin production by S. aureus in cured meats only after incubation for 2 days at 30°C, and after 3 days at 22°C.

Bologna pH also had a highly significant effect on growth of test organisms (Figures 1 to 5). After incubation at 30°C for 25 h, all organisms except C. perfringens grew

on high pH bologna. None of the pathogens, with the possible exception of S. aureus, grew on low pH bologna. The amount of growth (relative to the inoculum level), on high pH bologna, ranged from a 10^6-fold increase for S. aureus, and a 10^5-fold increase for B. cereus, to less than a 100-fold increase for S. typhimurium and enteropathogenic E. coli. S. aureus increased by less than 10^4-fold on pH 5.5 bologna, and declined on pH 5.1 bologna. Low pH not only prevented growth, but appeared to cause death of some test organisms. S. aureus declined on pH 5.1, but not on pH 5.5 bologna (Figure 1); B. cereus declined on pH 6.1 bologna, with lower recovery on pH 5.5 than on pH 6.1 product (Figure 4); C. perfringens declined more rapidly on low than on high pH samples (Figure 5). The decline appeared to be independent of incubation temperature for S. aureus and B. cereus, but not for C. perfringens.

The degree of significance that can be attached to the apparent death of S. aureus, B. cereus and C. perfringens is limited by the reliability of the counts, at low numbers per plate. In general, recoveries equivalent to less than a 3-fold relative population increase for S. aureus, S. typhimurium and B. cereus, and recoveries equivalent to less than a 0.3-fold relative population increase for enteropathogenic E. coli and C. perfringens were represented by colony counts of less than 30 colonies per plate (Appendix III). Counts of less than 30 colonies per plate

are subject to statistical unreliability when only duplicate counts are made.

Published reports considering the effects of pH on pathogen growth in cured meats are limited. A minimum growth pH of 5.0-5.1 has been reported for S. aureus in aerobically incubated, cured meats (10,105,114). Jensen (76) reported that S. aureus had a prolonged lag phase at pH 5.8. Genigeorgis et al. (42) observed inhibition of S. aureus in hams (up to 9.2% brine) at pH 5.6 during storage at 30°C, and at pH 5.3 during storage at 10°C. In this study, S. aureus experienced partial inhibition at pH 5.5 during incubation at 30°C. The lower salt concentration in bologna probably accounted for the decreased inhibition. S. typhimurium inoculated at 10^1-10^3 organisms per gram into vacuum packaged bologna (pH near 6.0) was reported to survive for several weeks at 7°C and to multiply slightly at 18°C and 24°C (33). In this study, neither growth nor death were observed for S. typhimurium at pH 6.1 or lower. Indications were that decline of S. typhimurium might have occurred after 25 h at 30°C in low pH bologna (Figure 2), but the range of counts obscured the significance of this observation. Hobbs (64) reported that C. perfringens was inhibited by a pH of 6.1 or less. The response of C. perfringens to pH in this study supported this observation. Examples of enteropathogenic E. coli and B. cereus responses to pH in cured meats were not reported in the literature.

The effect of age (i.e. competition) appeared less significant than the effects of pH and storage time-temperature. All organisms, except C. perfringens, grew on both age types of bologna after 25 h at 30°C. C. perfringens did not grow on either product type. Enteropathogenic E. coli (Figure 3) and possibly S. typhimurium (Figure 2) were the only test organisms affected by the age of the product. There was a 1,200- to 6,300-fold relative increase in enteropathogenic E. coli, and a 50- to 640-fold relative increase in S. typhimurium on new bologna. On old bologna, relative increases were 0- to 74-fold and 0.5- to 80-fold, respectively. The results for these two pathogens supported the observation of Heiszler et al. (62) that Gram negative bacteria could not compete successfully with the natural flora of cooked cured meats. The lack of increased inhibition of S. aureus and B. cereus in the older product was surprising. Goepfert et al. (47) reported that B. cereus was inhibited by the normal saprophytic flora in most foods, and that Group D streptococci in particular, antagonized B. cereus. Reports of S. aureus inhibition in mixed cultures are numerous (28,30,36,44,90,113,128). Lactic acid bacteria were reported to be especially effective inhibitors of S. aureus (28,30,44). Low lactic acid bacteria and Group D streptococcus counts in Canada Packers' bologna might explain, at least partially, the lack of increased inhibition of S. aureus and B. cereus in old samples

(Appendix II). It also seemed possible that competitive inhibition occurred at low bacterial populations and that increasing these numbers resulted in only minor additional inhibition.

The wide range of counts for individual pathogens, which was occasionally observed with replicate samples, might be the result of variations in the levels of inhibitors. The composition of the bologna samples reported in Appendix II indicate that there were small variations in percent moisture (i.e. % brine), pH, and bacterial counts. It was not possible in this study to determine which, if any, of these variables were responsible for the occasional wide ranges of pathogen counts. For low pathogen counts, the ranges might be due to statistical unreliability of the counts.

Organoleptically (i.e. cursory odor and appearance), the samples showed varied deterioration. Gross changes were not observed in the new samples even after incubation at 30°C for 25 hours. Sandwiches made with new, high pH bologna appeared edible even after this incubation. Organoleptic changes were obvious with all other bologna types before significant pathogen growth occurred. Greening was observed in old (high pH) bologna after 25 hours at 21°C and 30°C. Low pH bologna developed a slightly sour odor after 25 hours at 30°C.

The temperature equilibration times for bologna in sandwiches, during incubation are given in Table 12. These results indicate that the bread did not significantly affect the rate of temperature change, and therefore did not affect the potential for pathogen growth.

Table 12. Temperature equilibration times for bologna sandwiches in incubators.

Time (h)	Bologna temperature (°C)		
	4°C Incubator	21°C Incubator	30°C Incubator
0.0	16.0	15.0	18.0
0.5	8.0	18.0	27.0
1.0	6.0	19.5	28.5
1.5	5.5	20.0	29.0
2.0	4.5	20.5	29.5
2.5	4.5	21.0	30.0
3.0	4.5		
3.5	4.5		
4.0	4.0		

Chapter V. Summary and Conclusions

The microbial content and pH of vacuum packaged, sliced bologna in the Edmonton marketplace was highly variable. No single organism, or group of organisms, consistently predominated the bologna microflora. Of the organisms enumerated, lactic acid bacteria and Group D streptococci were most often observed in large numbers, sometimes accounting for a major portion of the total counts. Lactic acid bacteria showed the best correlation with pH. However, neither microbial counts nor pH were sufficiently reliable parameters that the characteristics to bologna in the marketplace could be predicted. High Group D streptococcus counts probably resulted from contamination of the product during slicing and packaging, followed by storage temperature abuse, as opposed to direct fecal contamination. S. durans are generally expected to be the predominating Group D streptococci in processed foods (119). However, not only S. durans, but also S. faecium and S. faecalis, were isolated from bologna samples. As a result, the high Group D streptococcus counts should be viewed with some concern. Group D streptococci are thought to have food poisoning potential (4,12,94,134). In addition, because 60% of the samples have counts above 100 Group D streptococci per gram, indications were that many samples have been exposed to considerable storage temperature abuse. Comparison of total counts at the three incubation temperatures supported this

conclusion.

Manufacturer and age of product had a definite effect on the microbial content and pH of the bologna samples. This might reflect different manufacturing practices, different product formulations (e.g. different fermentable carbohydrate concentrations) or different microflora predominating in the different manufacturing plants. The lower pH and higher bacterial counts observed in Gainers' product, and the higher pH and lower bacterial counts in Canada Packers' product made these ideal products for studying food poisoning potential of bologna in sandwiches. There was a marked interaction effect between manufacturer and age of product, with Canada Packers' product having unexpectedly high pH even with extended storage. The effects of retail display type on bacterial count and pH of bologna could not be clearly defined.

The survey results indicated that safety of vacuum packaged bologna was not affected adversely by high bacterial counts. Pathogen levels in these bologna samples were low, usually undectable, indicating either a low incidence of contamination or a failure of pathogens to survive in the vacuum packaged product (even when the pH was high). Bacterial standards, at least as regards total counts, therefore, are not meaningful for vacuum packaged bologna.

Safety of aerobically incubated bologna was evaluated in the inoculation study. Pathogens inoculated into bologna sandwiches required 8 hours incubation at 30°C, on high pH samples, for significant growth to occur. C. perfringens failed to grow, and in fact, declined on all bologna types. The rate of decline increased with increasing incubation temperature. All pathogens, except S. aureus were inhibited completely at or below pH 6.1. S. aureus showed reduced growth at pH 5.5 and was inhibited completely at pH 5.1. Only Gram negative pathogens appeared to be affected adversely by increased competition from the natural flora. Gram negative test organisms did not develop to the same extent as S. aureus and B. cereus, even on new (high pH) bologna, indicating, as suggested by Heiszler et al. (62), selection against Gram negatives. Organoleptically, only new (high pH) bologna remained acceptable after 25 h at 30°C. Bread provided bologna with only minor insulation from temperature change.

The inhibitory mechanism(s) in bologna are complex, and were not completely elucidated by this study. Even under the most favorable combination of pH, competition, oxygen partial pressure and storage temperature, there was no significant pathogen growth for more than 8 hours. Additional factors appeared to be critical to overall inhibitory capacity of bologna. Salt, or low water activity,

may be involved. S. aureus and B. cereus, the two most salt tolerant test organisms, showed the best ability to grow. Increased lag phase has been reported at low water activity (34,137). Inhibition by even low levels of competition (28), or by nitrite decomposition products (71,106,107) could also be involved. Additional studies would be required to determine the effects of these variables on pathogen growth in bologna or other cured meats.

To conclude, bologna in sandwiches could only be a vehicle of food poisoning in certain, almost unrealistic, situations. Only bologna contaminated with S. aureus, B. cereus, enteropathogenic E. coli or possibly salmonellae, exposed to highly abusive storage (i.e. more than 8 h at 30°C), and having low initial bacterial counts, and high initial pH appears to have food poisoning potential. The use of acid condiments (such as mayonnaise) probably would reduce this potential.

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Appendix I - Examples of Bologna Formulations (81)

Formulation A	Bologna		
	Ingredients	Amount	
		lb	oz
	whole-carcass beef	60	
	regular pork trimmings	40	
	ice	25	
	salt	2	12
	sugar		8
	ground white pepper		4
	coriander		1
	mace		1
	sodium erythorbate		0.85
	sodium nitrite		0.25
Formulation B	Bologna (Milk Powder)		
	regular pork trimmings	60	
	carcass beef	40	
	ice	25	
	dry skim-milk	4	
	salt	2	12
	sugar	1	
	white pepper		2
	coriander		1
	mace		1
	sodium erythorbate		0.85
	sodium nitrite		0.25
Formulation C	Bologna (Imitation)		
	beef weasand meat	30	
	beef trimmings	25	
	pork tongue trimmings	20	
	beef cheeks	10	
	pork stomachs	10	
	pork fat	5	
	ice	30	
	wheat flour	14	
	salt	3	
	sugar	1	
	white pepper		4
	garlic powder		1
	sodium nitrite		0.25

Appendix II - Profiles of bologna used for sandwich inoculation study

	Sample					
	1	2	3	4	5	6
Age (wks)	<1	<1	<1	5	5	5
Age Category	New	New	New	Old	Old	Old
pH	6.4	6.5	6.7	6.4	6.6	6.7
pH Category	N/A	N/A	N/A	High	High	High
Moisture (%)	59.4	58.0	58.7	57.3	56.0	55.8
Standard Plate Count[1] 4°C	1.0E3	1.2E2	2.8E2	3.2E7	2.9E8	2.5E6
21°C	5.5E3	1.8E2	2.2E2	3.2E7	2.6E8	2.0E6
36°C	5.6E3	4.3E4	3.0E2	3.5E6	1.2E8	2.8E5
Coliforms						
Presumptive	7.5E0	0.1E0	1.1E0	2.4E2	0.4E0	2.3E0
Confirmed	7.5E0	0.1E0	0.1E0	9.3E0	0.1E0	0.1E0
Completed	0.1E0	0.1E0	0.1E0	0.1E0	0.1E0	0.1E0
Fecal *E. coli*	1.0E0	1.0E0	1.0E0	1.0E0	1.0E0	1.0E0
NAP count	1.0E0	1.0E0	1.0E0	3.5E2	1.0E0	1.0E0
KF count	2.3E3	2.0E1	5.0E0	1.3E3	1.1E4	1.0E0
M. thermosphactum	1.0E1	1.0E1	1.0E1	3.0E7	5.8E6	1.3E6
Salmonella spp	-	-	-	-	-	-
B. cereus	5.0E1	1.0E1	1.0E1	1.0E1	1.0E1	1.0E1
MSA count	9.5E2	1.0E1	1.0E1	1.4E3	>1.0E6	2.5E4
C. perfringens	5.0E0	1.0E0	1.0E0	5.0E0	1.0E0	1.0E0

[1] E = x10* where * = integer immediately following E.

Appendix II continued:

	Sample				
	7	8	9[1]	10[2]	11[3]
Age (wks)	5	5	5	3	2
Age Category	N/A	N/A	N/A	N/A	N/A
pH	5.1	5.3	6.1	6.3	5.5
pH Category	Low	Low	Inter	Inter	Low
Moisture (%)	53.5	54.0	53.2	54.0	55.2
Standard Plate Count[4] 4°C	1.6E8	1.6E5	6.6E7	2.4E6	1.0E1
21°C	1.1E8	5.8E7	9.9E7	2.5E6	9.5E7
36°C	1.6E7	5.0E7	1 .0E8	2.0E6	1.8E8
Coliforms					
Presumptive	4.6E0	4.3E0	2.1E2	2.4E1	3.9E1
Confirmed	2.4E0	0.4E0	0.1E0	2.1E0	0.1E0
Completed	0.1E0	0.1E0	0.1E0	0.1E0	0.1E0
Fecal *E. coli*	1.0E0	1.0E0	1.0E0	1.0E0	1.0E0
NAP count	1.0E0	2.2E4	1.0E0	3.2E5	1.1E8
KF count	2.5E1	1.1E3	1.0E0	4.0E2	3.0E6
M. thermosphactum	1.0E4	2.5E2	1.0E1	7.8E5	1.0E1
Salmonella spp	-	-	-	-	-
B. cereus	1.0E1	1.0E1	1.0E1	1.0E1	1.0E1
S. aureus	1.0E0	1.0E1	1.0E1	1.0E1	1.0E1
MSA count	2.0E5	1.2E3	1.0E4	8.0E3	3.5E4
C. perfringens	1.0E0	1.0E0	1.0E0	1.0E0	1.0E0

[1] Results of Sample #9 are included with low pH bologna results for all organisms except *S. aureus*. *S. aureus* results are included with high pH bologna graph.
[2] *S. aureus* was the only organism inoculated onto this sample. Results are included in high pH bologna graph.
[3] *S. aureus* was the only organism inoculated onto this bologna.
[4] E = x10* where * = integer immediately following E.

Appendix III - Growth of Pathogens Required to Yield 30 Colonies on Media Plates

A 3 fold population increase on a 25 g slice which was inoculated with 500 organisms per gram represents 1500 organisms per gram. In analyzing the bologna, the 25 g slice was homogenized with 99 ml of diluent, and 0.1 ml was plated onto S. aureus, S. typhimurium and B. cereus media. On a wt/vol basis the number of organisms delivered per plate of media was:

(1500 org/g x 25 g x 0.1 ml/plate)/124 ml = 30 org/plate

A 0.3 fold population increase was required for enteropathogenic E. coli and C. perfringens because 1.0 ml rather than 0.1 ml was plated.